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## THE HOST RANGE AND BEHAVIOR OF THE ORDINARY TOBACCO-MOSAIC VIRUS[1]

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Although tobacco mosaic was described as early as 1886, the probable host range of the virus concerned has remained obscure. With the discovery of mosaic diseases in various other species, a strong tendency persisted on the part of some to attribute these to the virus of tobacco mosaic, or a somewhat modified form of this virus. Even though good evidence of the existence of different mosaic viruses began to accumulate about 1916, it was hardly sufficient for a decade to counteract this tendency. The more recent evidence of virus specificity based upon additional methods of identification has now enabled more reliable determination of host ranges to be made.

The ordinary tobacco-mosaic virus (*tobacco virus 1*) (23) has, in recent years, generally been regarded as limited to the Solanaceae. Confirmed evidence has existed for some time, however, that this virus is transmissible to 2 genera outside this family. This has suggested the possibility of a wider host range and the need of more complete information on this subject in relation to both the classification of plant viruses and the development of control measures. With this purpose in view a systematic study of the host range was undertaken, selecting for the tests widely separated plant species. The results secured show that *tobacco virus 1* is capable of developing in certain species through a wide range of plant families, with no appreciable modification of the virus itself in the different hosts.

### REVIEW OF LITERATURE

Mayer (28) first reported that the tobacco-mosaic disease could be transmitted mechanically from infected to healthy tobacco plants. The first proof that tobacco-mosaic virus is transmissible to other solanaceous genera (*i.e.*, Lycopersicon) was reported by Clinton (6) in 1908. Allard (1), in 1914, re-

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ported tobacco mosaic as communicable to a number of other solanaceous species. All of his efforts, however, to communicate the disease to plants of families other than the Solanceae gave negative results. The following plants were inoculated by the most rigorous methods without any visible symptoms of the disease appearing: sheep sorrel, white clover, red clover, peanut, soy bean, cauliflower, peppergrass, rib-grass, dead nettle, wood sorrel, garden lettuce, and pokeweed. Similarly, Duggar and Armstrong (10) and Johnson (23) failed to transmit the tobacco-mosaic virus to *Phytolacca decandra* L. Clinton and McCormick (7) and E. M. Johnson (19) also reported failure to transmit this virus to certain other non-solanaceous plants.

Following the work of Allard, numerous species were reported, however, as showing mosaic symptoms. In 1922 Dickson (8) reviewed the literature and listed 96 species on which mosaic had been found. Of these species, 30 belong to the Solanaceae, 20 to the Cucurbitaceae, 18 to the Leguminosae, and 7 to the Gramineae, the other 21 hosts being distributed through 17 families. Dickson recognized the possibility that there might be different mosaic diseases involved; however in his review he made no distinction, listing only those plants on which mosaic had been found.

Elmer (11), in 1922, reported that cross-inoculation experiments showed the mosaic diseases of the Cucurbitaceae, Solanaceae, and Leguminosae to be intertransmissible. In 1925 he further reported (12) the mosaic virus transmissible among species belonging to different families and orders. Fifteen interfamily and 11 interorder transmissions were obtained. Results of a similar nature were reported by Palm (30), Palm and Jochems (31), Walker (36), and Doolittle and Walker (9). It is evident that at this time the specificity of viruses was not generally recognized or accepted, even though somewhat prior to and during this period evidences of differences in host range and symptomatology were being presented by Allard (2), Schultz and Folsom (33), Fernow (13), and Johnson (20, 21).

The danger of confusion of plant viruses in host-range studies is evident from Johnson's (23) description of 11 different viruses on tobacco, as differentiated on the basis of several factors applicable as tests

Fernow (13), in 1925, described his mosaic A, which, as nearly as can be determined, was due to the ordinary tobacco-mosaic virus, as transmissible to *Martynia louisiana,* Mill. of the family Martyniaceae. The susceptibility of this nonsolanaceous plant to this virus was confirmed later by Beale (3), and Hoggan (15). This is, then, the first nonsolanaceous species definitely shown to be susceptible to the ordinary tobacco-mosaic virus. In 1930 Price (32) tested 77 commercial varieties of *Phaseolus vulgaris* L. and found that certain varieties develop local necrotic lesions when juice, con-

taining virus of ordinary tobacco mosaic, is rubbed onto the upper surfaces of the leaves, thus establishing this species, also, as a host.

## MATERIALS AND METHODS

One hundred and twenty-one species representing 40 widely separated families were included in this study. The selection of these species was based first upon securing a wide family distribution, and secondly upon the availability of seed; hence the use of a number of common crop, ornamental, and weed plants. Previous mention in literature of susceptibility of certain species to mosaic, especially to tobacco mosaic, rendered the inclusion of such species desirable. The majority of seed was obtained from the following seed companies: L. L. Olds, Madison, Wisconsin; H. G. Hastings, Atlanta, Georgia; and Theodore Payne, Los Angeles, California. The weed seeds were secured from A. L. Stone, Agronomy Department, University of Wisconsin. All species used were, as nearly as could be determined, correctly named. The seed was sown in greenhouse flats and the seedlings transplanted to fertile compost soil in 4-in. pots.

The ordinary tobacco-mosaic virus (*tobacco virus 1*) was the same as that used in a previous investigation by Johnson and Grant (25). The original source was maintained by frequent direct inoculations to healthy young tobacco plants. These stock plants for the virus were kept in a special chamber and precautions were taken to avoid contamination with other viruses. All plants tested for susceptibility were inoculated with extracts obtained from these stock plants.

The experiments were carried on during the fall, winter, and spring in 2 greenhouses, one of which was maintained at a temperature of 80° to 90° F., and the other at 65° to 70° F. During the summer of 1932 infected plants were grown out of doors. This afforded an opportunity to study symptom expression of various hosts under field conditions.

Holmes' wiping method of inoculation (16) was employed throughout the present investigation. Leaf material was crushed in a mortar and the extracted juice strained through cheesecloth. A cheesecloth pad, saturated with extract, was rubbed over the entire upper surface of the leaves inoculated. The lower leaves only of the young plants were inoculated and the plants were then allowed to grow for 2 to 3 weeks or more to allow sufficient time for the distribution of the virus in case of infection. Inoculations with extracts from the noninoculated leaves were then made to *Nicotiana tabacum* L. and *N. glutinosa* L. to determine the presence or absence of the virus. All plants inoculated with the virus were tested for susceptibility in this manner. Furthermore, additional plants of susceptible species were repeatedly tested, and the symptom expression noted in comparison with noninoculated controls.

The distribution and relative concentration of the virus were determined by making separate extracts from top, middle, and basal leaves and inoculating to 5 leaves of *Nicotiana glutinosa* plants. The number of lesions appearing after 3 to 5 days was then recorded. The methods used for determining the thermal death point, tolerance to dilution, and longevity *in vitro* of virus obtained from different hosts were the same as those described by Johnson (23, 25). In this investigation it was necessary to take special precautions to obtain extracts with as high virus content as possible. Details of other methods used are presented in the text.

## EXPERIMENTAL RESULTS

### Host Range

The host range studies were limited to nonsolanaceous species. Over 2,000 flowering plants, representing 40 families, 104 genera, and 121 species, were inoculated with the ordinary tobacco-mosaic virus. A summary of the results secured is given in table 1. No infection was obtained on 76 per cent of the species tested. It is difficult to attach any great significance to this fact, however, since proof that a particular species is immune from the virus would no doubt require a larger number of trials under a greater variety of environmental conditions than was made in these tests. The main difficulties encountered in the tests were the consequence of the erratic spread of the virus in some hosts, rendering determination of infection laborious, and the different environmental conditions required for satisfactory vegetative development of some species. While the negative results obtained by the inoculation of species grown under similar environmental conditions are of some interest in terms of percentage of immune species, they are not believed to be of sufficient value to list in full in this paper.

No infection was obtained on any monocotyledonous plants, although 16 different species representing 5 families were tested. Special attention was given to the inoculation of plants in the family Gramineae, such as: corn, rye, wheat, barley, oats, various millets, sudan grass, sorghum, feterita, and sugar cane.

One hundred and five dicotyledonous species representing 35 families were inoculated. Negative results were obtained on all species tested in 21 of these families. In some of these families several species were used; as, for example, 6 species in the Labiatae, 6 in the Cucurbitaceae, and 4 in the Malvaceae. On the other hand, positive infection resulted from the inoculation of 29 species included in the following 14 families: Polygonaceae, Chenopodiaceae, Aizoaceae, Ranunculaceae, Cruciferae, Leguminosae, Umbelliferae, Convolvulaceae, Polemoniaceae, Hydrophyllaceae, Boraginaceae, Scrophulariaceae, Martyniaceae, and Compositae.

In most of these families infection was not secured on all species tested.

TABLE 1.—*Results of inoculating certain species of various plant families with the ordinary tobacco-mosaic virus*

| Family name | Number species tested | Number plants inoculated | Number species infected |
|---|---|---|---|
| Gramineae | 12 | 390 | 0 |
| Araceae | 1 | 5 | 0 |
| Commelinaceae | 1 | 15 | 0 |
| Liliaceae | 1 | 10 | 0 |
| Cannaceae | 1 | 5 | 0 |
| Polygonaceae | 2 | 79 | 1 |
| Chenopodiaceae | 2 | 147 | 2 |
| Amaranthaceae | 2 | 24 | 0 |
| Nyctaginaceae | 1 | 5 | 0 |
| Aizoaceae | 1 | 20 | 1 |
| Portulacaceae | 1 | 10 | 0 |
| Carophyllaceae | 1 | 5 | 0 |
| Ranunculaceae | 3 | 44 | 1 |
| Papaveraceae | 1 | 10 | 0 |
| Cruciferae | 7 | 180 | 2 |
| Leguminosae | 13 | 96 | 1 |
| Geraniaceae | 1 | 5 | 0 |
| Tropaeolaceae | 1 | 5 | 0 |
| Euphorbiaceae | 1 | 5 | 0 |
| Balsaminaceae | 1 | 5 | 0 |
| Malvaceae | 4 | 65 | 0 |
| Violaceae | 1 | 5 | 0 |
| Lythraceae | 1 | 15 | 0 |
| Onagraceae | 1 | 21 | 0 |
| Umbelliferae | 2 | 37 | 1 |
| Plumbaginaceae | 1 | 10 | 0 |
| Apocynaceae | 1 | 14 | 0 |
| Convolvulaceae | 4 | 68 | 2 |
| Polemoniaceae | 2 | 8 | 1 |
| Hydrophyllaceae | 5 | 90 | 5 |
| Boraginaceae | 2 | 30 | 1 |
| Verbenaceae | 1 | 20 | 0 |
| Labiatae | 6 | 67 | 0 |
| Scrophulariaceae | 7 | 171 | 6 |
| Martyniaceae | 1 | 10 | 1 |
| Plantaginaceae | 1 | 5 | 0 |
| Valerianaceae | 1 | 10 | 0 |
| Cucurbitaceae | 6 | 60 | 0 |
| Campanulaceae | 1 | 10 | 0 |
| Compositae | 19 | 363 | 4 |
| | 121 | 2144 | 29 |

In the Compositae only 4 out of 19, in the Leguminosae 1 of 13, and in the Convolvulaceae 2 of the 4 species tested were found susceptible to infection. In the family Hydrophyllaceae, however, all 5 species inoculated became infected.

The 29 species found susceptible to infection are: buckwheat, *Fagopyrum esculentum* Gaertn.; garden and sugar beet, *Beta vulgaris* L.; Swiss chard, *B. vulgaris* var. *cicla* L.; spinach, *Spinacia oleracea* L. var. King of Denmark; New Zealand spinach, *Tetragonia expansa* Murr.; larkspur, *Del-*

*phinium consolida* L.; mustard, *Brassica alba* Rabenh.; turnip, *B. rapa* L.; bean, *Phaseolus vulgaris* L. var. Green Pod Refugee; carrot, *Dacus carota* var. *sativa* DC.; morning glory, *Ipomoea tricolor* Cav.; cypress vine, *Quamoclit pennata* Bojer; phlox, *Phlox drummondii* Hook.; California blue bell, *Phacelia whitlavia* Gray, *P. campanularia* Gray, *P. grandiflora* (Benth.) Gray, *P. tanacetifolia* Benth., *P. parryi* Torr.; Chinese forget-me-not, *Cynoglossum amabile* Stapf. and Drummond; mullein, *Verbascum thapsus* L.; Kenilworth ivy, *Linaria cymbalaria* (L). Mill.; snapdragon, *Antirrhinum majus* L.; foxglove, *Digitalis purpurea* L.; Maryland figwort, *Scrophularia marylandica* L.; beard-tongue, *Penstemon barbatus* Roth.; unicorn plant, *Martynia louisiana* Mill.; zinnia, *Zinnia elegans* Jacq.; French marigold, *Tagetes patula* L.; cape marigold, *Dimorphotheca aurantiaca* DC.; tassel flower, *Emilia sagittata* (Vahl.) DC.[3]

## Symptom Expression and Type of Infection

The descriptions of symptoms and type of infection, as typical for the 29 nonsolanaceous hosts, must be, in a sense, only relative. The natural variations in environment and other circumstances, such as age and vigor of the host plant inoculated, may modify both the distribution of the virus and the symptom expression. Certain of the hosts, however, repeatedly developed remarkably specific symptoms and virus distribution and may be used to illustrate the various types of infection secured (Table 2).

California blue bell, *Phacelia whitlavia,* showed systemic infection very similar in type to that generally recognized as occurring with ordinary tobacco mosaic on tobacco, *Nicotiana tabacum.* Mottling, malformation, and chlorosis were the predominating manifestations (Fig. 1, A, B.). In other species, such as larkspur and Chinese forget-me-not, there was occasionally more stunting of the plant and considerable necrosis (Fig. 2, B.). The virus was generally found distributed throughout the plant, and these hosts were considered, therefore, as being systemically infected. Spinach, also, was placed with this group of hosts on the basis of symptom expression.

In contrast to the above species, which were found to be systemically infected, others showed a localized type of infection, the virus apparently not spreading much beyond the inoculated portion. Garden beet, Swiss chard, and New Zealand spinach first exhibited symptoms of yellowing only at the point of entrance of the virus. These yellow areas usually extended until the entire leaf was chlorotic. The leaf finally became necrotic and sometimes dropped off. The symptoms appearing on Green Pod Refugee

[3] Credit should be given to Dr. Maurice B. Lindford for being first to secure infection with the ordinary tobacco-mosaic virus on *Emilia sagittata,* while performing certain experiments at Wisconsin during the summer of 1931.

TABLE 2.—*A comparison of type infection and symptom expression obtained with ordinary tobacco mosaic on nonsolanaceous host plants*

| Type of infection | Host species | Host family | Number of plants[a] | Symptom expression[b] |
|---|---|---|---|---|
| Systemic | *Phacelia whitlavia* (California blue bell) | Hydrophyllaceae | 30<br>27 | M–MF–s |
| | *Phacelia parryi* | " | 20<br>20 | M–MF–s |
| | *Phacelia campanularia* | " | 10<br>10 | M–MF |
| | *Phacelia grandiflora* | " | 20<br>20 | Y–M mf |
| | *Phacelia tanacetifolia* | " | 10<br>10 | Y–m–n |
| | *Delphinium consolida* (larkspur) | Ranunculaceae | 28<br>28 | Y–M–n |
| | *Cynoglossum amabile* Chinese forget-me-not) | Boraginaceae | 25<br>25 | M–mf–s |
| | *Martynia louisiana* (unicorn plant) | Martyniaceae | 10<br>7 | M–mf–s |
| | *Spinacia oleracea* (spinach) | Chenopodiaceae | 55<br>44 | Y–M–s–n |
| Local | *Beta vulgaris* (garden beet) | Chenopodiaceae | 35<br>30 | Y–N |
| | *Beta vulgaris* (sugar beet) | " | 5<br>5 | Y–N |
| | *Beta vulgaris* var. *cicla* (swiss chard) | " | 52<br>44 | Y–N |
| | *Tetragonia expansa* (New Zealand spinach) | Aizoaceae | 20<br>20 | Y–N |
| | *Phaseolus vulgaris* (green pot refugee bean) | Leguminosae | 5<br>5 | N |
| Erratic | *Emilia sagittata* (tassel flower) | Compositae | 45<br>37 | MS–m |
| | *Linaria cymbalaria* (Kenilworth ivy) | Scrophulariaceae | 35<br>29 | Ms–y–m–mf |
| | *Phlox drummondii* (phlox) | Polemoniaceae | 18<br>12 | M–y |
| | *Digitalis purpurea* (foxglove) | Scrophulariaceae | 25<br>25 | Y–m |
| | *Verbascum thapsus* (mullein) | " | 22<br>12 | Y–m |
| | *Ipomoea tricolor* (morning glory) | Convolvulaceae | 39<br>12 | Y–n |
| | *Tagetes patula* (French marigold) | Compositae | 55<br>39 | Y–m–n |
| | *Zinnia elegans* (zinnia) | " | 55<br>23 | Y–m |
| | *Fagopyrum esculentum* (buckwheat) | Polygonaceae | 64<br>46 | M–MF–n–s |

TABLE 2.—(*Continued*)

| Type of infection | Host species | Host family | Number of plants[a] | Symptom expression[b] |
|---|---|---|---|---|
| Undetermined | *Antirrhinum majus* (snapdragon) | Scrophulariaceae | 35/6 | MS–Y |
| | *Penstemon barbatus* (beard-tongue) | " | 44/8 | MS–Y |
| | *Scrophularia marylandica* (Maryland figwort) | " | 9/5 | MS–y |
| | *Quamoclit pennata* (cypress vine) | Convolvulaceae | 21/3 | Y–n |
| | *Daucus carota* var. *sativa* (carrot) | Umbelliferae | 25/1 | M–n |
| | *Brassica alba* (mustard) | Cruciferae | 65/15 | M–N |
| | *Brassica rapa* (turnip) | " | 20/5 | M–N |
| | *Dimorphotheca aurantiaca* (Cape marigold) | Compositae | 20/6 | N–mf |

[a] In this and succeeding tables, upper figure represents number of plants inoculated; lower figure, number of plants infected.

[b] M—mottling; MF—malformation; S—stunting; Y—yellowing; N—necrosis; MS—masked symptoms. Small type indicates mild form of symptoms.

bean were essentially of the same type of necrotic lesion as described by Price (32).

During the course of this investigation other species were found in which the type of infection could not be definitely classed as either systemic or localized. The uncertain behavior of this group of species with respect to infection has led us to place it in a third category designated as "erratic." Since this study was undertaken Holmes (18) also has reported that in certain solanaceous hosts the virus spreads erratically from the inoculated leaf. Essentially the same method as that used by Holmes has been applied in the study of certain of the nonsolanaceous hosts characterized by irregular reaction to inoculation. Leaves from top, middle, and basal portions of such plants were crushed separately in mortars and the extracts, so obtained, used for inoculation to *Nicotiana glutinosa*. The total number of lesions appearing after 3 to 5 days was counted and the data are presented in table 3. These counts not only serve to show the presence or absence of the virus in the various parts of the plant, but indicate as well its relative concentration.

Ordinarily, tassel flower and Kenilworth ivy did not show any symptoms, though some mottling and chlorosis could be induced by partial defoliation. The results obtained on virus distribution indicated that the virus was present in higher concentration in the basal than in the top leaves.

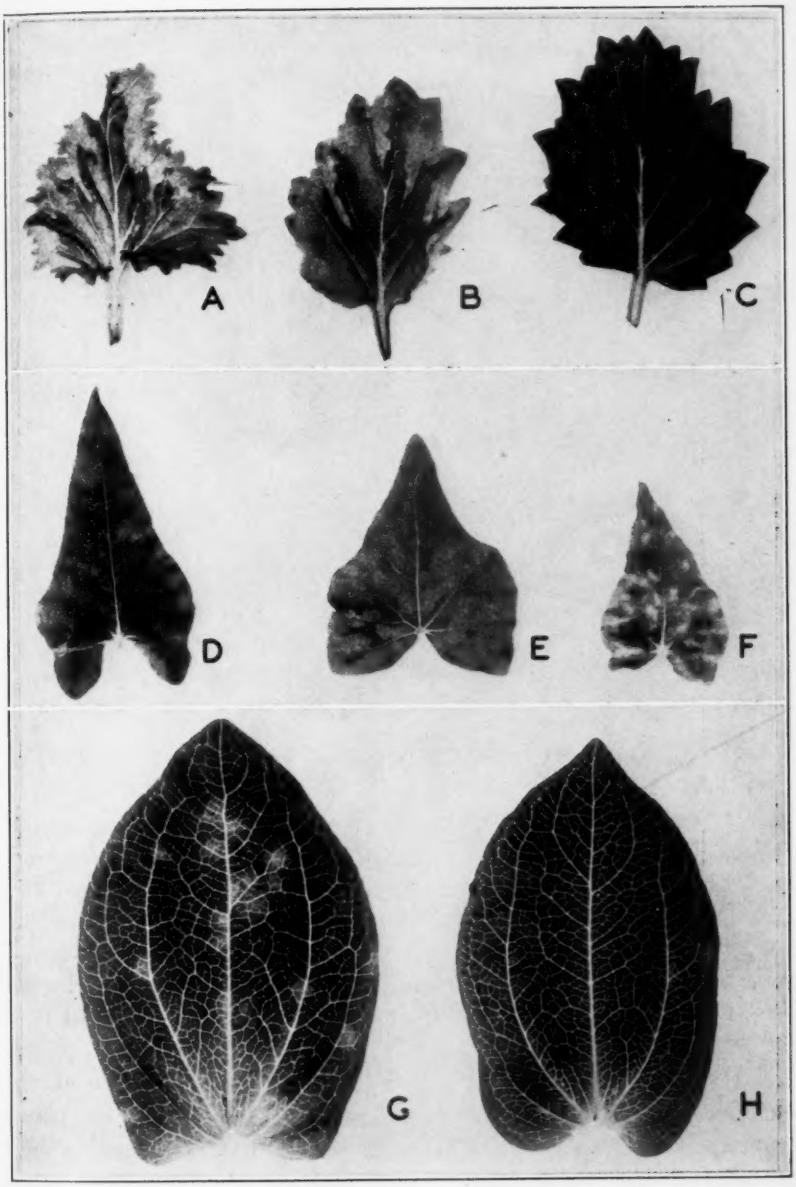


FIG. 1. Leaf symptoms of the ordinary tobacco mosaic on certain nonsolanaceous species. A. and B. Infected leaves of *Phacelia whitlavia*, showing mottling, malformation, and chlorosis. C. Leaf from noninfected control. D–F. Infected leaves of *Fagopyrum esculentum* (buckwheat). G. Infected leaf of *Zinnia elegans*, showing chlorotic spots resulting from spread of the virus from lower inoculated leaves. H. Leaf from noninfected control.

FIG. 2. Symptoms of ordinary tobacco mosaic on certain nonsolanaceous plants. A. Healthy *Cynoglossum amabile* (Chinese forget-me-not). B. Early stunting and malformation due to virus infection. C. Healthy *Tagetes patula* (French marigold). D. Infected plant showing stunting and malformation of leaflets.

Foxglove showed definite symptoms of chlorosis on the inoculated leaves. The youngest leaves did not at first show symptoms, although, as they became older, yellow spots appeared which coalesced to form either chlorotic or mottled areas. The distribution and concentration of the virus in this species seemed to be quite definitely related to the symptom expression. No virus was detected in the youngest leaves, while the highest concentrations were found in the older leaves.

In morning glory and mullein, the virus apparently had difficulty in

TABLE 3.—*Comparison of tobacco-mosaic virus content of basal, middle, and top leaves from various plants by means of inoculation to Nicotiana glutinosa*

| Infected host | Period following inoc. (weeks) | Av. number of lesions on 5 leaves | | |
|---|---|---|---|---|
| | | From basal leaf | From middle leaf | From top leaf |
| *Emilia sagittata* (tassel flower) | 3 | 198 | 185 | 0 |
| | 3 | 205 | 193 | 0 |
| | 3 | 421 | 264 | 0 |
| | 3 | 271 | 342 | 2 |
| | 10 | 136 | 4 | 0 |
| | 10 | 417 | 18 | 32 |
| *Linaria cymbalaria* (Kenilworth ivy) | 8 | 437 | 425 | 370 |
| | 9 | 240 | 210 | 67 |
| | 9 | 164 | 231 | 116 |
| | 9 | 184 | 27 | 4 |
| | 11 | 326 | 3 | 2 |
| | 11 | 258 | 205 | 7 |
| *Digitalis purpurea* (foxglove) | 7 | 610 | 340 | 0 |
| | 8 | 800 | 205 | 0 |
| | 8 | 456 | 700 | 0 |
| | 8 | 650 | 177 | 0 |
| | 8 | 346 | 189 | 0 |
| | 16 | 315 | 19 | 0 |
| *Tagetes patula* (French marigold) | 1 | 265 | ..... | 0 |
| | 4 | 532 | 499 | 519 |
| | 4 | 670 | 451 | 556 |
| | 4 | 650 | 0 | 0 |
| | 7 | 128 | 0 | 0 |
| | 8 | 373 | 204 | 292 |
| | 8 | 184 | 0 | 0 |
| | 8 | 238 | 226 | 282 |
| *Zinnia elegans* (zinnia) | 3 | 162 | ..... | 0 |
| | 3 | 7 | ..... | 0 |
| | 4 | 46 | ..... | 0 |
| | 4 | 223 | ..... | 133 |

passing from the inoculated leaves. The inoculated portion of the plant usually became chlorotic, and when the virus did pass from this portion it was found only in localized yellow spots or small areas on leaves immediately above. Older plants did not show symptoms on the top or youngest leaves.

In French marigold the virus was sometimes confined to the basal leaves and at other times was found in relatively high concentrations in all parts

of the plant tested. Although the symptom expression was variable, the inoculated leaves usually showed some chlorosis. The plant might be stunted in development and malformed, as shown in Figure 2, D, or it might develop mild symptoms of scattered chlorotic and necrotic spots. Leaf development in these spots was partially inhibited and, as a result, the leaf was slightly distorted.

The distribution of the virus in zinnia was particularly variable; it might be localized, causing only local lesions, or it might spread to other leaves, resulting in the appearance of small round yellow spots (Figure 1, G.). The spots sometimes remained yellow for a few days and finally disappeared or sometimes coalesced to form a large yellow blotch, which either remained the same color or changed to a light green.

In buckwheat the virus apparently passed readily from the inoculated leaf and, at first, produced what appeared to be typical systemic infection (Fig. 1, D–F), though, as the plant continued to grow, the symptoms became less marked in the new growth and the concentration of the virus decreased. Occasionally, necrosis developed in the stem and produced a lesion that caused distortion or death of the entire plant.

In this group of plants typified by erratic infection it was found that the symptom expression was extremely variable, ranging from masked symptoms to yellowing with some mottling and necrosis. The results secured from the testing of various plant parts showed definitely that there was considerable variation in the distribution of the virus. The significance of this is perhaps most strikingly brought out by comparison with results obtained by Holmes (17), who studied the local and systemic increase of tobacco-mosaic virus on *Nicotiana tabacum*.

A few of the nonsolanaceous plants inoculated exhibited a considerable degree of resistance to infection. In some species the percentage of plants infected was small, rendering determination of the type of infection difficult, although the presence of the virus in the infected plants was definitely proved by reinoculation to *Nicotiana glutinosa* and *N. tabacum*. During the early part of this investigation several series of snapdragon plants were inoculated, but infection was secured on only two. These plants did not develop definite symptoms. The presence of the virus in some shoots and not in others was determined by reinoculation to *N. glutinosa*. One of the infected snapdragon plants was grown in the garden during the summer of 1932 and seed was obtained from this plant. When this seed was planted and the young plants inoculated, 4 of 10 tested became infected.

Observations made on 8 infected beard-tongue plants indicated that these behaved in a manner similar to snapdragon. Cypress vine showed some yellowing of the basal leaves, and these contained virus, but the tops were apparently virus-free.

The study of the type of infection obtained in Maryland figwort was limited by the available seed supply. However, the plants tested showed that the virus might reach the top, though it was not found there in such high concentration as in the lower leaves. There were no definite symptoms, although the lower leaves finally became yellow.

One carrot plant, which became infected, showed mottling in all the leaves and these later became necrotic. This plant was inoculated in the greenhouse and later transplanted to the garden. The presence of tobacco-mosaic virus in this plant was tested several times by reinoculation to both tobacco and *Nicotiana glutinosa* and positive results were obtained in each case. Repeated attempts to secure further infection on carrots failed.

Mustard and turnip included in 3 separate trials during the winter of 1931 developed symptoms of marked mottling followed by complete necrosis of the plants. The seed source used in these first trials was exhausted and the plants grown from other seed have never shown infection. The infections secured on cape marigold were the result of inoculations made in a number of different trials. The symptoms consisted of necrosis of the midribs of the leaves with subsequent curling and dwarfing of the plant.

Since, in most cases, the number of plants infected in this latter group of species was so low as to preclude satisfactory determination of type of infection, these have been listed in table 2 under "undetermined" type of infection.

The low percentage of infection obtained from repeated inoculation of certain nonsolanaceous hosts is of considerable interest and significance. Evidently, no "true symptomless carriers" of the virus were found. In every case where the virus was recovered from the species inoculated, symptoms were expressed at some stage of development, although partial masking of symptoms was not uncommon in many species.

## Properties of the Virus in Different Hosts

*Thermal Death Point.* The thermal death point or inactivation point of ordinary tobacco-mosaic virus from tobacco generally is accepted as being close to 90° C. for a 10-minute treatment. Some variation is to be expected as a consequence of various circumstances, even though the conditions of the trials are made as constant as possible. According to McKinney (29) higher dilutions of the virus may lower the thermal death point several degrees. In a previous investigation Johnson and Grant (25) determined the thermal death point of the tobacco-mosaic virus in undiluted extracts from various solanaceous plants in which the virus occurred systemically. They concluded that in the species tested the thermal death point for this virus was just below 90° C., but that certain host species concerned might vary it as much as 5° C.

In the present investigation, due to the fact that the virus spread erratically in certain host plants, it was necessary to determine the location of the virus in these plants so that the possibility of dilution with the plant juice would be reduced to a minimum. The inoculated portion of the plants was not employed as a source of inoculum in any of the tests reported in tables 4 and 5. All the data on inoculation to *Nicotiana glutinosa*

TABLE 4.—*Results of thermal death-point determinations on the tobacco-mosaic virus from different host species following inoculation to Nicotiana tabacum*

| Source of inoculum | Inoc. control | Temperature °C. (10 min.) | | | |
|---|---|---|---|---|---|
| | | 80 | 85 | 90 | 95 |
| *Nicotiana tabacum* (tobacco) | 10/10 | 10/10 | 10/10 | 10/2 | 10/0 |
| *Phacelia whitlavia* (California blue bell) | 15/15 | 15/14 | 15/10 | 15/0 | 15/0 |
| *Spinacia oleracea* (spinach) | 20/13 | 20/19 | 20/2 | 20/5 | 20/0 |
| *Digitalis purpurea* (foxglove) | 15/15 | 15/15 | 15/15 | 15/0 | 15/0 |
| *Fagopyrum esculentum* (buckwheat) | 10/10 | 10/10 | 10/9 | 10/0 | 10/0 |
| *Zinnia elegans* (zinnia) | 15/15 | 15/15 | 15/6 | 15/0 | 15/0 |
| *Linaria cymbalaria* (Kenilworth ivy) | 15/12 | 15/13 | 15/10 | 15/6 | 15/0 |
| *Tagetes patula* (French marigold) | 15/15 | 15/15 | 15/15 | 15/1 | 15/0 |

presented in table 5 are based upon inoculation with the same samples of extract as those used to inoculate *N. tabacum*, as shown in table 4. However, not all the treated extracts inoculated to tobacco (Table 4) were inoculated to *N. glutinosa* because of the limited amount of space available in the greenhouse and the lack of sufficient *N. glutinosa* plants at the time the tests were made.

In table 4, using the number of *Nicotiana tabacum* plants infected as a criterion, it is shown that in extracts from *Phacelia whitlavia* infected with tobacco mosaic the virus was not greatly inactivated at 85° C., but was destroyed at 90° C. Similar results were secured with foxglove, buckwheat, and zinnia. In the case of spinach, Kenilworth ivy, and French marigold, the virus was not entirely inactivated at 90° C., a result similar to that frequently obtained with virus extract from tobacco.

TABLE 5.—*Results of thermal death-point determinations on the tobacco-mosaic virus from different host species following inoculation to Nicotiana glutinosa*

| Source of inoculum | Number trials | Av. number of lesions on 5 leaves | | | | |
|---|---|---|---|---|---|---|
| | | Inoc. control | Temperature °C. (10 min.) | | | |
| | | | 80 | 85 | 90 | 95 |
| *Nicotiana tabacum* (tobacco) | 2 | 602 | 433 | 264 | 1 | 0 |
| *Phacelia whitlavia* (California blue bell) | 2 | 208 | 47 | 9 | 0 | 0 |
| *Spinacia oleracea* (spinach) | 3 | 22 | 69 | 1 | 1 | 0 |
| *Digitalis purpurea* (foxglove) | 3 | 120 | 136 | 103 | 0 | 0 |
| *Fagopyrum esculentum* (buckwheat) | 1 | 425 | 287 | 51 | 0 | 0 |
| *Zinnia elegans* (zinnia) | 3 | 184 | 98 | 2 | 0 | 0 |
| *Linaria cymbalaria* (Kenilworth ivy) | 2 | 224 | 252 | 89 | 0 | 0 |
| *Tagetes patula* (French marigold) | 2 | 208 | 163 | 108 | 0 | 0 |

The data presented in table 5, using the number of lesions on *Nicotiana glutinosa* as the criterion, suggest that in extracts of *Phacelia whitlavia* infected with tobacco mosaic the virus was, to some extent, inactivated at 85° C., and completely inactivated at 90° C. Similar results were obtained with foxglove, buckwheat, zinnia, Kenilworth ivy, and French marigold. The results secured with spinach, however, are distinctly different. With the nontreated extract, a small number of lesions were produced on *N. glutinosa* and a larger number were produced with extracts heated at 80° C. Inactivation of the virus was not obtained at 90° C., as was indicated for all the other hosts except tobacco. While the results obtained from control inoculations made with the various nontreated plant extracts show considerable variation in the number of lesions produced, it is difficult to attach much significance to these variations except in the case of such marked differences as occur in spinach. This exception will be considered in more detail later.

The data presented (Tables 4 and 5) support the generally accepted conclusion that the thermal death point of the tobacco-mosaic virus is close to 90° C. for a 10-minute treatment, regardless of the host in which it

develops. Although *Nicotiana glutinosa* is of value in detecting differences in relative concentration of the virus, it apparently is not so sensitive as *N. tabacum* in the detection of very low concentrations of virus.

*Tolerance to dilution.* The results secured by diluting the infectious extract from various species with different amounts of water followed by inoculation to tobacco are presented in table 6. A relatively high per-

TABLE 6.—*Results of dilution trials with the tobacco-mosaic virus from different host species following inoculation to Nicotiana tabacum*

| Source of inoculum | Dilution of virus | | | | | |
|---|---|---|---|---|---|---|
| | None | 1 to 100 | 1 to 1,000 | 1 to 10,000 | 1 to 100,000 | 1 to 1,000,000 |
| *Nicotiana tabacum* (tobacco) | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/8 |
| *Phacelia whitlavia* (California blue bell) | 15/15 | 15/15 | 15/15 | 15/15 | 15/15 | 15/11 |
| *Spinacia oleracea* (spinach) | 25/22 | 25/23 | 25/25 | 25/25 | 25/25 | 25/19 |
| *Digitalis purpurea* (foxglove) | 15/15 | 15/15 | 15/15 | 15/15 | 15/15 | 15/9 |
| *Fagopyrum esculetum* (buckwheat) | 15/15 | 15/15 | 15/15 | 15/11 | 15/6 | 15/5 |
| *Zinnia elegans* (zinnia) | 15/15 | 15/15 | 15/15 | 15/14 | 15/7 | 15/1 |
| *Linaria cymbalaria* (Kenilworth ivy) | 24/22 | 24/20 | 24/16 | 24/15 | 24/11 | 24/6 |
| *Tagetes patula* (French marigold) | 10/10 | 10/10 | 10/10 | 10/7 | 10/6 | 10/3 |

centage of infection was secured on tobacco plants inoculated with *Phacelia whitlavia* extract diluted to 1 to 100,000 and 1 to 1,000,000. Similar results were obtained with the dilutions of extracts from infected spinach, foxglove, and tobacco. In the case of buckwheat and Kenilworth ivy only fair percentages, and with French marigold and zinnia low percentages of infection were secured at these higher dilutions. These differences may be explained partly on the basis of virus distribution and concentration in the various original extracts. It has been shown that the virus spreads erratically in French marigold, Kenilworth ivy, zinnia, and buckwheat, and it is possible that the original extracts from these hosts did not contain the virus in such highly concentrated condition as might be expected from tobacco or

*Phacelia whitlavia.* However, it is significant that some infection was obtained from all hosts used at the highest dilution of 1 to 1,000,000.

Inoculations of some of the above diluted extracts also were made to *Nicotiana glutinosa,* and the average number of lesions appearing on 5 leaves is recorded in table 7. With diluted extracts of *Phacelia whitlavia,*

TABLE 7.—*Results of dilution trials with the tobacco-mosaic virus from different host species following inoculation to Nicotiana glutinosa*

| Source of inoculum | Number trials | Av. number of lesions on 5 leaves | | | | | |
|---|---|---|---|---|---|---|---|
| | | Dilution of virus | | | | | |
| | | None | 1 to 100 | 1 to 1,000 | 1 to 10,000 | 1 to 100,000 | 1 to 1,000,000 |
| *Nicotiana tabacum* (tobacco) | 2 | 670 | 463 | 233 | 149 | 46 | 17 |
| *Phacelia whitlavia* (California blue bell) | 2 | 493 | 339 | 115 | 81 | 32 | 3 |
| *Spinacia oleracea* (spinach) | 5 | 16 | 22 | 46 | 67 | 13 | 12 |
| *Digitalis purpurea* (foxglove) | 2 | 237 | 162 | 134 | 113 | 22 | 3 |
| *Fagopyrum esculentum* (buckwheat) | 2 | 356 | 119 | 18 | 3 | 2 | 1 |
| *Zinnia elegans* (zinnia) | 2 | 166 | 41 | 11 | 2 | 1 | 1 |
| *Linaria cymbalaria* (Kenilworth ivy) | 2 | 150 | 169 | 84 | 71 | 2 | 1 |
| *Tagetes patula* (French marigold) | 2 | 240 | 134 | 56 | 41 | 15 | 1 |

the average number of lesions decreased as the amount of dilution increased. Similar results were obtained with diluted extracts from infected foxglove, buckwheat, zinnia, French marigold, and tobacco. These data support the conclusion that the number of lesions appearing on *N. glutinosa* may be used in a general way to indicate the relative amounts of virus present. However, there were apparently certain cases in which this was not true. The average number of local lesions appearing after inoculation with undiluted Kenilworth ivy extract was less than that obtained from extract diluted to 1 to 100. In this instance the results obtained were, as nearly as could be determined, due to the toxic effect of the Kenilworth ivy juice on *N. glutinosa,* as indicated by necrosis in the leaf areas inoculated. Similar toxic action resulted from extract from healthy Kenilworth ivy.

The toxicity of the juice of this plant was not always apparent, and it is believed that this toxicity may in some way be associated with environmental conditions existing at the time of or subsequent to inoculation.

The results obtained by inoculation with spinach extracts were strikingly different from those secured with extracts of other hosts. In the case of spinach extract the average number of lesions was always low, even though the undiluted extract was obtained from leaves showing good mottling. Similarly, fewer lesions were produced on *Nicotiana glutinosa* with the undiluted extract than at dilutions of 1 to 1,000 to 1 to 10,000. The apparently deleterious influence of spinach on the tobacco mosaic virus seemed to be of sufficient significance to warrant further study.

*Influence of plant extracts on the virus.* Allard (1), in 1914, carried out experiments to determine whether the sap of mosaic plants could be rendered innocuous by mixing it with different portions of the sap of what were believed to be immune species. He used extracts of healthy *Nicotiana viscosum* (*N. glutinosa*) and *N. glauca* and found that the treated virus in no instance lost its infectious properties. In 1925 Duggar and Armstrong (10) also studied the effect of treating the virus of tobacco mosaic with the juices of various plants. They found that the juice of pokeweed, *Phytolacca decandra*, effectively inactivated the agency of tobacco mosaic in a relatively short time (15 to 18 hours). Even when the virus was diluted only in a proportion of 1 part virus extract to 5 parts pokeweed juice, the inhibition was complete. Inactivation also was caused by the juice of Jimson weed, a solanaceous plant, where the latter was in relatively high concentration, and, to some extent, also, by the juice of geranium. On the other hand, cotton, squash, potato, sweet potato, and apple exerted no injurious influence at the concentrations tested. They state that it seemed conceivable that inactivation of the mosaic virus might be due to adsorption.

In the present investigation the healthy plants selected as sources of extracts for treatment of the tobacco-mosaic virus were chosen for various specific reasons. Spinach plants were used because of the unusual behavior noted in the previous tests (Tables 4, 5, 6, and 7). *Nicotiana glutinosa*, beet, Swiss chard, and bean were employed because of the fact that they exhibited only a localized type of infection when inoculated with the ordinary tobacco-mosaic virus. Snapdragon was used because of the low percentage of infection obtained (Table 2) from inoculation with the ordinary tobacco-mosaic virus. *Phytolacca rigida* Small, was chosen as a source of extract because of its close relationship to *P. decandra* which has previously been shown to have an inactivating or adsorptive effect upon the virus. Dilution with water and with extracts from healthy tobacco plants were made as controls.

The method employed was essentially as follows: leaves from healthy plants were ground in a meat chopper, the juices strained through cheese-

cloth, and 9-cc. portions placed in test tubes. The extract containing virus was obtained by crushing leaves of infected tobacco plants in a mortar and straining the juice through cheesecloth. A 1-cc. portion of this infective extract was placed in each test tube containing the 9 cc. of healthy plant juice. The test tubes were then shaken for about 2 minutes, and 2-cc. portions of the mixture were poured off and used to inoculate 5 leaves of *Nicotiana glutinosa* plants. The remaining 8 cc. of mixtures were placed in the ice box at 4° C. until the following day (18 to 24 hours later), when the tubes were removed, shaken, and a 2-cc. sample of each poured off and again inoculated to *N. glutinosa*. The number of local lesions appearing on 5 leaves after a period of 3 to 5 days was recorded, and the average number of lesions for all trials is shown in table 8. In each case a portion of the

TABLE 8.—*The influence of various plant extracts on the tobacco-mosaic virus, as tested by inoculation to Nicotiana glutinosa*

| Source of healthy juice | Number trials | Av. number of lesions on 5 leaves | |
|---|---|---|---|
| | | Time of inoculation | |
| | | Immediately | After 1 day |
| *Nicotiana tabacum* (tobacco) | 3 | 682 | 668 |
| *Nicotiana glutinosa* | 3 | 460 | 639 |
| *Antirrhinum majus* (snapdragon) | 3 | 322 | 388 |
| *Phaseolus vulgaris* (bean) | 3 | 232 | 224 |
| *Spinacia oleracea* (spinach) | 3 | 16 | 12 |
| *Beta vulgaris* (beet) | 3 | 32 | 26 |
| *Beta vulgaris* var. *cicla* (Swiss chard) | 3 | 9 | 11 |
| *Phytolacca rigida* | 3 | 10 | 5 |
| Water control | 3 | 724 | 750 |

extracts of the healthy plants also was inoculated to *N. glutinosa* as an added check on the absence of the virus from the plants. In all tests of these extracts no local lesions were obtained. Furthermore, these juices caused no macroscopically visible toxic effects on the inoculated *N. glutinosa* leaves.

One-cc. portions of ordinary tobacco-mosaic-virus extract from tobacco

diluted with 9 cc. of distilled water produced about 700 local lesions on *Nicotiana glutinosa* (Table 8). Similar results were secured with dilutions of virus with extracts of healthy tobacco plants. The average number of lesions obtained from dilutions with extracts of *N. glutinosa,* snapdragon, and bean was somewhat lower. The number secured from dilutions with the extracts of spinach, beet, Swiss chard, and *Phytolacca rigida* was significantly lower. It is also interesting to note that the results obtained from inoculations made immediately after mixing and those made one day later were not essentially different. In any one series of tests the amount of virus present in each 1-cc. portion should be approximately the same because the portions were obtained from a single sample of juice. The average of the 3 trials as presented in table 8 is representative of the results obtained in each series of tests.

It is apparent from the above results that extracts from beet, Swiss chard, spinach, and *Phytolacca rigida* have a deleterious effect on the infectiousness of the ordinary tobacco-mosaic virus. The low number of local lesions obtained on *Nicotiana glutinosa* with extracts from infected spinach (Tables 4, 5, 6, and 7) may not, therefore, be attributable merely to a low concentration of the virus in this nonsolanaceous host.

*Resistance to Aging in Vitro.* Extracts of tobacco containing the ordinary tobacco-mosaic virus are known to retain their infectious nature for long periods of time. It was thought desirable to determine whether the length of time that the virus could resist aging would be materially reduced in extracts obtained from infected nonsolanaceous hosts. The 8 species selected for sources of infectious extract were: California bluebell, *Phacelia whitlavia, P. grandiflora, P. parryi,* Kenilworth ivy, foxglove, spinach, tassel flower, and Chinese forget-me-not. Extract from an infected tobacco plant was used as a control. The aging test was made by placing 10 cc. of the extracts in stoppered test tubes for a period of 3 months in a darkened drawer in a greenhouse where the temperature usually ranged between 80 to 90° F. Following this treatment, inoculations were made to tobacco plants to determine the presence of the virus. All the extracts employed in this test were found to have retained their infectious properties for the 3-month period.

## Host Influence on Virus Attenuation

In 1925 Carsner (5) found that passage of the virus of curly-top of sugar beet through certain resistant plants resulted in attenuation of the virus. Recently, Lackey (27) reports that attenuated virus of sugar beet curly-top, obtained by passage through *Chenopodium murale* L., was restored to its original virulence by passage through *Stellaria media* (L.) Cyr. Johnson and his coworkers (22, 24) have shown that several different

environmental factors may bring about attenuation of the tobacco-mosaic virus. They found that virulence of this virus, as measured by symptom expression, was not restored by repeated transfer through tobacco plants. A case of attenuation of the tobacco-mosaic virus as a result of passage through Martynia also has been reported from this laboratory (25).

These instances of attenuation suggested the desirability of testing the effect of other nonsolanaceous hosts on the tobacco-mosaic virus. The 5 species selected for study were: *Phacelia whitlavia,* larkspur, buckwheat, tassel flower, and French marigold. Successive transfers of the ordinary tobacco-mosaic virus were made through a series of young plants of each species. With each transfer of a portion of the infectious extract to the respective species, inoculations were also made to tobacco for the purpose of noting possible modification of symptoms. At the same time inoculations were made to *Nicotiana glutinosa* for the purpose of readily checking on the specificity and the amount of virus transferred in each case. Controls were simultaneously inoculated with virus extract obtained from tobacco.

In this manner the virus was transferred through 4 successive series of *Phacelia whitlavia,* larkspur, buckwheat, tassel flower, and through 3 series of French marigold plants. No definite evidence of attenuation was secured in these trials. On the basis of these preliminary tests it is concluded that the ordinary tobacco-mosaic virus is at least not regularly nor readily attenuated by passage through nonsolanaceous host plants.

## Cytological Observations

Microscopic examinations of the tissues of tobacco plants infected with the ordinary tobacco-mosaic virus have been made by numerous investigators. Two types of cell inclusions, namely, x-bodies and striate material, have been described in association with tobacco mosaic on tobacco. In 1927, Hoggan (14) found these 2 types of cell inclusions to be a constant feature of the ordinary tobacco-mosaic virus on other solanaceous host plants that developed definite symptoms of mottling or chlorosis. In 1931, Hoggan (15) transmitted the ordinary tobacco-mosaic virus to *Martynia louisiana* and reported the presence of characteristic cell inclusions in this host also.

The method employed in the present investigation was essentially the same as that described by Hoggan (14). Small portions of healthy and diseased plant tissues were cut out of the leaf lamina with a sterile safety-razor blade, placed immediately in the fixing fluid (100 parts 50 per cent alcohol, 6.5 parts Formalin, and 2.5 parts glacial acetic acid), and allowed to remain 24 to 48 hours. The usual procedure for embedding in paraffin was carried out. The material was sectioned at a thickness of 7μ in a

plane perpendicular to the leaf surface, and stained with Heidenhain's iron-alum haematoxylin.

The stained sections of infected tissue from chlorotic leaves of *Phacelia whitlavia* showed the presence of numerous x-bodies and some striate material, (Fig. 3, A, B). Bodies and material of similar form and size were found in chlorotic tissue of larkspur (Fig. 3, D), Kenilworth ivy, and foxglove. In spinach (Fig. 3, C), however, a lesser number of x-bodies and

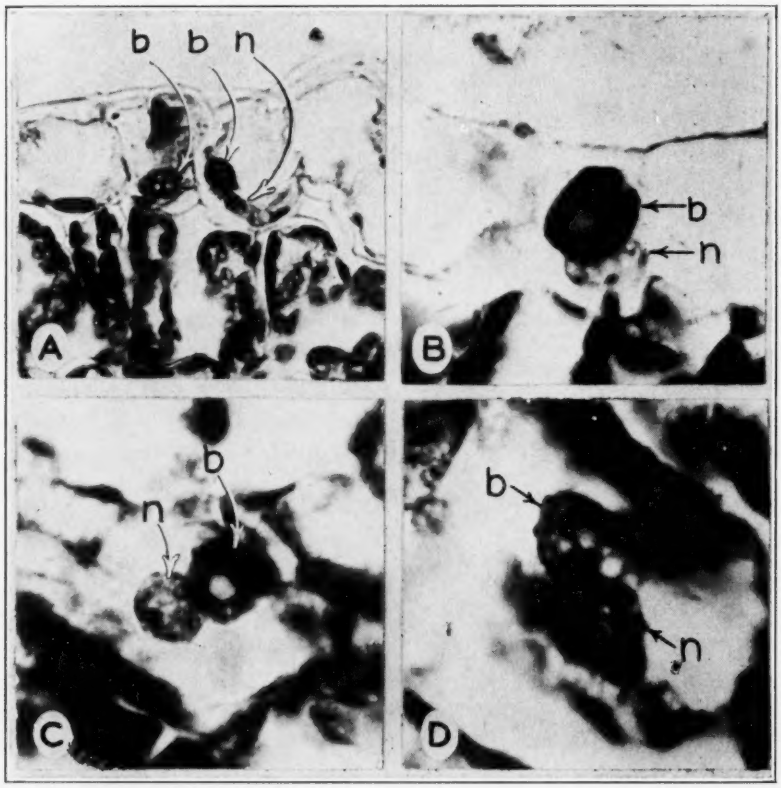


FIG. 3. Photomicrographs showing x-bodies (b), and nucleus (n), in leaf tissue of nonsolanaceous hosts infected with tobacco mosaic. A. *Phacelia whitlavia* (California blue-bell). ×700. B. *P. whitlavia*. ×1,600. C. *Spinacia oleracea* (spinach). × 1,750. D. *Delphinium consolida* (larkspur). ×1,600.

smaller amounts of striate material were observed, while in Maryland figwort larger quantities of striate material and only a few x-bodies were found. Examination of stained sections of comparable healthy tissues failed to show the presence of any such inclusions.

Observations of infected leaf tissue from buckwheat did not disclose

any typical inclusions, possibly because of the limited amount of material examined.

## DISCUSSION

Casual observation of plants in nature and in culture, as well as frequent reports in literature, testify to the common occurrence of viruses in the flowering plants. The greater interest now lies in the particular virus concerned in each case, rather than in the occurrence of the disease itself. The number of different viruses which exist in nature may not be large as compared with other groups of parasites, and it is likely that they will eventually be fairly well classified. A desirable preliminary to any classification scheme for plant viruses is a general understanding of the host range of each specific virus. With this information as a basis, the utilization of differential hosts should be simplified and methods of determination shortened.

Only a few viruses have previously been intensively studied from the point of view of host range. The outstanding examples are the viruses of aster yellows (26), tobacco ring-spot (37) and curly-top of sugar beet (4, 34, 35). These viruses have very extensive host ranges, whereas other viruses, though not intensively investigated (*e.g.*, mild mosaic and crinkle mosaic of potato), evidently are very limited in host range.

The experimental data presented in this paper will aid in clearing up some obscure conceptions relative to the classical tobacco-mosaic virus. The theoretical host range of this virus has passed through a succession of stages. While it was at first conceded only a very limited host range, it was later associated in a confusing manner with other viruses, which, as a group, were reported to be intertransmissible among species that are widely separated taxonomically (12). For a time these results were interpreted by some as applying to the ordinary tobacco-mosaic virus, whereas this virus was apparently only one of several that were being employed without distinction.

With the more general recognition of virus specificity, the host range was then believed to be limited to the Solanaceae (21). The present investigations show, however, that it may be transmitted experimentally to other widely separated families among dicotyledonous plants. Furthermore, the high percentage of infection secured (24 per cent) among the species tested suggests that still other species in other families will, when tested, prove susceptible.

On the whole, the 29 nonsolanaceous host species reported in this paper are not highly susceptible, but the variation in behavior in this respect is apparently not much greater than that to be found in the solanaceous family itself. The range of symptom expression in the susceptible non-

solanaceous species is also quite comparable to that described by Holmes (18) within the Solanaceae.

The behavior of the virus of ordinary tobacco mosaic with respect to its properties and the occurrence of the characteristic cell inclusions in several of the nonsolanaceous hosts add support to accumulating data on the specificity and stability of this virus under varying conditions. Further evidence of the stability of this virus is shown by the failure to obtain attenuation by passage through several series of widely separated nonsolanaceous hosts. The deleterious effect of the extracts of certain nonsolanaceous plants is not unusual, since a similar effect may be exercised by members of the Solanaceae, as in the case of *Datura stramonium* L. (10).

The results secured in this investigation also may eventually have some bearing upon the development of control measures for the disease caused by this virus. If wild or overwintering hosts should be found to play an important part in the origin and dissemination of the disease in local areas, it is evident that certain nonsolanaceous as well as solanaceous plants must be considered as probable "carriers."

## SUMMARY

The host range of the ordinary tobacco-mosaic virus, generally regarded as limited to certain species within the family Solanaceae, has been extended to include 29 species, representing 14 widely separated families.

Symptom expression in these nonsolanaceous plants is characterized by marked variations ranging from masked symptoms to typical chlorosis, mottling, malformation, necrosis, and stunting, alone or in combination. The distribution of the virus in these plants varies from typical systemic infection to localized infection or erratic spread from the inoculated portion.

The host species in which the virus developed did not radically influence the constancy of the properties of the virus as measured by thermal death point, tolerance to dilution, and aging *in vitro*.

The virus did not become attenuated by passage through several series of certain nonsolanaceous hosts.

In the case of spinach infected with tobacco mosaic, the virus appeared to exist in only very low concentration. Extract from healthy spinach plants, however, acted deleteriously on virus extracts from tobacco of high concentration. Consequently, low percentages of infection secured from certain susceptible species are evidently not necessarily a result of low concentrations of the virus in such hosts.

The presence in several nonsolanaceous hosts of intracellular bodies commonly associated with the ordinary tobacco-mosaic virus has been established cytologically.

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# DOWNY MILDEW OF TOBACCO[1]

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## INTRODUCTION

Downy mildew of tobacco, commonly known as blue mold, occurs ubiquitously throughout the entire area devoted to the culture of flue-cured tobacco. Observations of the past 3 seasons indicate that it is primarily a disease of seedlings. They indicate also that the disease will continue to appear in epiphytotic proportions every spring, although it may vary in destructiveness.

Nearly all investigations of downy mildew that have hitherto been made, have been conducted in Australia where this disease is endemic. The results of these investigations, in their most essential features, are summarized in a recent publication by Angell and Hill (2). The present studies deal with such matters as the life history and morphology of the pathogen, the source of inoculum in spring, the agencies of dissemination, the pathology of the disease, and the relation of weather conditions to development and spread. They are, in part, confirmatory of previous investigations in Australia.

## HISTORY OF THE DISEASE

Definite records of downy mildew on tobacco in Australia date from 1890, although it was probably present 30 or 40 years earlier (2). The first record of its occurrence within the United States is that by Farlow (9) who noted it on *Nicotiana glauca*, an introduced ornamental species, near San Diego, California, in 1885. He prophetically stated at that time that, "It is much to be feared that the disease which attacks *Nicotiana glauca* may sooner or later extend to cultivated tobacco. If this happens the injury to the tobacco will be very great . . . . If *N. glauca* and its parasite are once introduced into the Gulf States, the parasite might attack the tobacco grown there and then pass on to Virginia and the other states where tobacco is the most important crop." Partial fulfillment of this prophecy came in March, 1921, when the disease made its appearance in Gadsden County, Florida, and Decatur County, Georgia, on seedlings of shade-grown tobacco. In their account of this outbreak, Smith and McKenney (13) pointed out

[1] A cooperative investigation by the Departments of Botany and Chemistry, Duke University. The writers wish to express their gratitude for their cooperation to Dr. P. M. Gross and Mr. E. G. Moss, and to the several farm demonstration agents and interested growers for their courtesies.

that collections of mildew on cultivated tobacco seedlings had been made in Texas, in 1906, and that the disease may have been introduced into the Florida-Georgia area by way of California and Texas. The possible spread to Florida through gradual extension of its range is indicated by the collection of downy mildew on *Nicotiana bigelovii* in Nevada (15). Burger and Parham (7) expressed the opinion that downy mildew probably existed in Florida over a period of years prior to 1921. Smith and McKenney (13) also suggested the possibility of its introduction with mats from the East Indies. Palm (11) maintained that this source of introduction is improbable because (1) the mats are made in Borneo, in a section where tobacco is not grown, (2) the causal organism is not known to occur in the Dutch East Indies, and (3) the chance of contamination, en route, by contact of mats with tobacco from China and India, or in storage in Holland with tobacco from Brazil and Europe, is remote.

For some unknown reason, downy mildew did not again appear in epiphytotic proportions until the early spring of 1931, when it occurred throughout Florida, Georgia, and Louisiana. Before the end of the season for transplanting it had appeared in many sections of South Carolina, North Carolina, and Virginia. In 1932 it appeared on seedlings in these last-named states considerably earlier in the season, and spread to all tobacco seed beds throughout the entire Coastal Plain and Piedmont sections of the Atlantic seaboard, as far northward as Maryland. In 1933, the disease spread to the Burley-tobacco sections of Western North Carolina, and of Tennessee, and extended its range as far northward as Pennsylvania.

### SUSCEPTS

McAlpine (10), in early investigations of downy mildew in Australia, reported that the same organism that attacks cultivated tobacco occurs also on *Nicotiana suaveolens*, a species native to Australia. Adam[2] noted that *N. glauca*, *N. rustica*, and *N. longiflora* are also suscepts. Angell and Hill (2) are of the opinion that all species of Nicotiana are probably subject to the disease because of their observations that seedlings of the following species are readily attacked: *N. angustifolia*, *N. atropurpureum*, *N. calciflora*, *N. paniculata*, *N. campanulata*, *N. sylvestris*, *N. nudicaulis*, *N. repanda*, *N. caudigera*, *N. triangophylla*, *N. chinensis*, *N. glutinosa*, *N. acuminata*, *N. langsdorfii*, *N. laternia*, and *N. bigelovii*.

The writers' observations indicate that seedlings of all agronomic varieties of flue-cured tobacco are equally susceptible to downy mildew. In addition, they have observed the disease on seedlings of tomato, *Lycopersicum esculentum*, pepper, *Capsicum annuum*, and eggplant, *Solanum melongena*, when these species were being grown near tobacco seed beds

[2] Reported from Adam's unpublished notes by Angell and Hill (2).

or in the same seed beds with diseased tobacco seedlings. Similar observations, recorded in the *Plant Disease Reporter*[3] have been made by a number of investigators. Potted seedlings of these three solanaceous species, when inoculated with sporangia from diseased tobacco seedlings, become infected about a week after inoculation.

*Hyoscyamus niger,* when grown in beds among diseased tobacco seedlings, or when inoculated in pots under conditions favorable for infection of tobacco seedlings, remains free from attack. These observations accord with those of Angell and Hill (2). Potato, *Solanum tuberosum,* remained free from attack when artificially inoculated. *S. nigrum* and *S. carolinense,* two weeds of rather common occurrence in or near tobacco seed beds, are also apparently immune from downy mildew. Other immune solanaceous species include Jerusalem cherry, *S. pseudo-capsicum,* and apple of Sodom, *S. sodomaeum,* (Darnell-Smith (8)).

## APPEARANCE OF THE DISEASE

Downy mildew is primarily a seedling disease in the flue-cured tobacco-growing area. It usually appears about 2 weeks prior to the time that the seedlings are of size suitable for transplanting. It may, however, attack seedlings of any age, even those just emerging, observations that confirm those of Angell and Hill (2).

The symptoms of the disease, its rate of spread throughout the bed, and the severity of attack, are modified by such factors as weather conditions, age and vigor of the seedlings, location of beds with reference to exposure and soil and air drainage, density of the stand, and construction and management of the beds.

The most characteristic feature of the downy mildew is the presence of a dense, downy coating on the underneath portions of mature lesions on diseased leaves (Fig. 2). The presence of the disease is first indicated by the general aspect of beds. One may note, on observing a bed in which the disease is first beginning to appear, that small groups or patches of seedlings are yellowish green in color. There may be only one such patch or at most a few, even in beds several hundreds yards in area. These areas may appear in any portion of the seed bed but are most commonly present around stumps or in places in which the seedlings are making a subnormal growth. Closer inspection of yellowish green seedlings reveals that the tips of many of the upper leaves droop and are flaccid. At this stage the sporangia and sporangiophores of the causal fungus may not yet have appeared. The under surface of the lower leaves of such plants, however, may be partly or entirely covered with a downy felt. During periods favor-

[3] U. S. Dep. Agr., Bur. Pl. Indus. Pl. Dis. Rptr. **17**: 37–39. 1933.

able for the development of the pathogen, as during cool weather in which the sky is continuously overcast, the downy coating may appear on the upper leaf surface, also. This is of common occurrence on leaves in thickly sown beds or on the most shaded leaves. When, at this time, affected leaves are viewed from above, they show large, irregular, yellow patches or the leaves may be uniformly yellowish (Fig. 1). Large, dry, irregular areas

FIG. 1. Downy mildew on upper and lower surfaces of leaves of tobacco seedlings.

replace the yellow patches within a few hours on clear windy days. Under these conditions the general aspect of the seed bed is as of plants that have been scalded. This scalding is most pronounced when clear weather follows a cloudy period during which every seedling has become involved. The rapid collapse of seedlings is accompanied by a characteristic vile odor, quite like that of decaying cabbage.

Usually, small seedlings are killed outright, but the "bud leaves" of the larger ones remain apparently normal, even though all the lower leaves have collapsed. Such plants as survive may have recovered sufficiently within 2 weeks to be transplanted.

FIG. 2. Lower surface of lesion, enlarged, showing a profuse coating of the sporangial stage of *Peronospora nicotianae*.

Pittman (12) regards the name "blue mould" as inappropriate because the least conspicuous feature of the fungus is its blueness. When, however, fresh material is examined in early morning or when there has been a period characterized by rainy days with cold nights, the down has a pronounced violet tint. When the lesions have dried or after several hours of sunshine the fructifications, *en masse*, are grayish to pale brown.

If, after a brief period favorable for infection, a period of dry weather prevails, indefinite yellow specks appear on the upper leaf surface. These are abortive lesions, which may persist unchanged or their centers may become necrotic. The growth of the normal tissue surrounding these abortive lesions may result in the leaf surface becoming puckered or contoured. Such lesions may never bear a crop of sporangia if the weather remains dry and warm. It is for this reason that there seems to be no way of determining in advance, from the appearance of any given infected leaf, whether sporangia will appear and of prophesying the date of such an appearance.

During a period favorable for the development of the disease the collapse of infected leaves usually follows within a few hours the production of sporangia on those leaves. In some cases, though, daily crops of sporangia may be formed along the advancing margin of a given lesion.

Seedlings that have survived an attack of downy mildew and have developed new leaves, seen not to be subject to a second attack. This observation is supported by numerous field observations and by attempts to artificially inoculate seedlings that have recovered. No satisfactory explanation for the apparent immunity can be made at this time.

Downy mildew occurs in Australia on the leaves of mature plants and causes the formation of large lesions that may ultimately drop out(2). It may also appear there, during autumn, on the suckers, flower buds, flowers, and capsules. It is improbable, because of the relation of weather conditions to the development of the causal fungus, as will be pointed out later in this report, that downy mildew will attack the growing crop in the field in the flue-cured area.

## THE CAUSAL ORGANISM

*Morphology.* The fungus that causes downy mildew of tobacco has, in common with many other downy mildews, two life-cycle stages, *i.e.*, sporangia and oospores. The sporangia are borne at the surface of the lesions prior to the death of the tissues and the oospores, within the dead leaf tissues.

The sporangia are elliptical to oval bodies 15–28 by 12–18 μ, and are densely filled with pigmented, granular material and scattered oil droplets. They are borne on dendritic, dichotomously branched sporangiophores, which branch 4 to 8 times, and terminate in curved, acute apices (Fig. 3, A). The sporangiophores occur either singly or in small groups. They range in height from 400 to 750 μ The diameter of their bases is 10 to 12 μ. The length of the stalk approximates two-thirds the height of the sporangiophore.

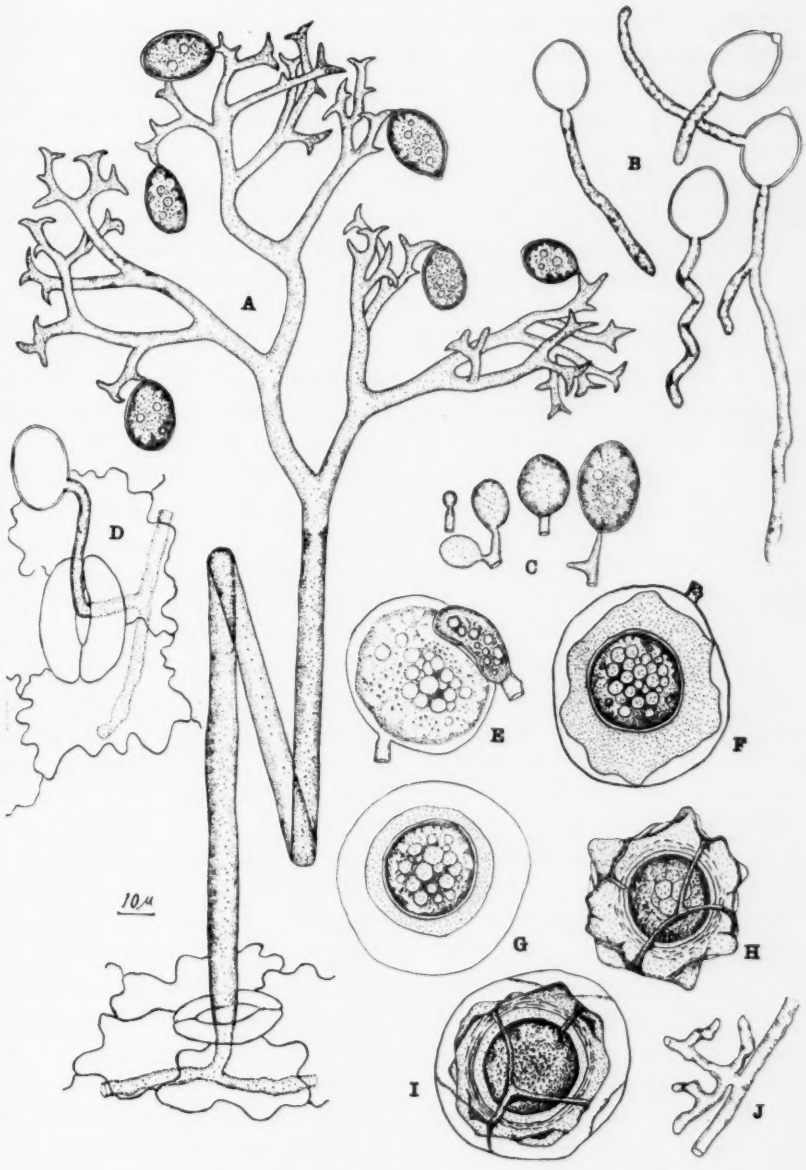


FIG. 3. A. Sporangiophore of *Peronospora nicotianae* from which most of the sporangia have been dislodged. B. Germinating sporangia. C. Stages in the matinal development of sporangia at the apices of sporangiophore. D. Infection tube entering a stoma. E. Young spherical oogonium with a clavate antheridium applied to it. F. and G. Stages in the development of the oogonium, in which the hyaline tunic and the brown periplasm and oospore have been differentiated. H. and I. Mature oospores with thickened wall. J. Haustorium.

Sporangial formation has been studied by stripping off the epidermis of infected leaves and examining under a microscope. Sporangiophores arise as branches from the intercellular hyphae and emerge from the stomates. They soon become bifurcately branched, and, as development continues, a dendritic structure with blunt, dichotomous tips arises. These enlarge into bulbous processes, (Fig. 3, C), and by the time they have attained approximately half their ultimate size, a septum delimits the young sporangium from the sporangiophore. At this stage the fructifications are colorless, but, because they cover the lesions so copiously, the leaf surface appears to be covered with a white down. By the time the sporangia have attained their mature size, their color is distinctly violet, and the sporangiophore tips have become curved and taper-pointed.

As the result of a series of all-night vigils it was found that sporangial production is matinal. It begins with the advent of dawn and the sporangia are mature by sunrise. If the weather is cloudy maturity is somewhat delayed. Conceivably, this process could continue throughout dark days, but this is not known to be true.

Mature oospores have been observed in material collected from such widely separated localities as Lumberton, Wilson, Townsville, Oxford, and Apex, North Carolina, and Blackstone, Virginia. They occur within the tissues of old leaves that have collapsed and remained in contact with the soil, also in leaves kept in moist chambers 4 or 5 days after the tissues have become necrotic. When such tissue is examined with low-power magnification, the oospores appear as bright, reddish brown spheres embedded within the decaying leaf, and may be so abundant that 100 or more can be seen in a microscopic field.

Young oogonia are spherical structures, hyaline, but filled with an abundance of refractive granules and oil globules (Fig. 3, E). The antheridium is a bluntly clavate structure, somewhat concave on the surface applied to the oogonium (Fig. 3, E). Apparently, coincident with fertilization, the ooplasm becomes differentiated into a thin-walled, dense, pale brown, central oosphere with a less dense periplasm and an outer, hyaline envelope (Fig. 3, F and G). As the oospore matures, its wall thickens and the depth of color increases until it finally becomes reddish brown. Meanwhile, the periplasm becomes contoured into low, blunt elevations and ridges (Fig. 3, H and I). The hyaline, outer tunic collapses with desiccation and resumes its normal form when moistened. Oogonia range from 60 to 85 μ in diameter and the oospores from 45 to 75 μ. It has not been possible to accomplish the germination of these oospores.

*Identity.* The organism that causes downy mildew of tobacco has hitherto been designated *Peronospora hyoscyami* deBary (6, p. 123–124), an organism described in 1863, on *Hyoscyamus niger*. It would appear

FIG. 4. Loci of early outbreaks of downy mildew, originating from seed beds located on old sites.

from the observations of Angell and Hill (2), and the previously mentioned inoculation experiments of the writers, that *H. niger* is immune from the pathogen that causes downy mildew of tobacco. Since downy mildews are all obligate parasites that are rather restricted in suscept range, it seems more reasonable, therefore, to regard the downy mildew of tobacco as specifically distinct from *P. hyoscyami*. It does not appear probable that immunity of *Hyoscyamus niger* from the tobacco fungus can be explained by physiological modification or changed virulence of the pathogen.

In 1891 Spegazzini (14, p. 36) described *Peronospora nicotianae* on *Nicotiana longiflora* from collections made in Argentina. Unfortunately, Spegazzini's type specimens are not available for comparison. The variability of the downy mildew on cultivated tobacco suggests that a direct comparison of it with type specimens of *P. nicotianae* might still leave a doubt as to their identity. This variation is shown in the summary, which follows. Angell and Hill (2) pointed out that the maximum, minimum, and average measurements of sporangia for the organism in Australia are each 50 per cent greater in January than in May. Their length measurements lay between 12 and 34 μ, and the breadth between 10 and 20 μ, with 22 by 16 μ as the average dimensions. Spegazzini's measurements are 18–20 by 9–12 μ, and ours are 15–28 by 12–18 μ.

There is, in Australia, also a seasonal variation in size of sporangiophores. Angell and Hill's (2) measurements, in the growing season, show a range in height of from 589 to 1024 μ, in spread of crown of from 230 to 435 μ and in number of times the sporangiophores branch of from 5 to 8; in winter the height is 243 μ, the breadth 89 μ, and the number of times branched, 4. Spegazzini's height measurement is 250 to 500 μ, and ours 400 to 750 μ.

Angell and Hill (2) state that oospores are rarely found in Australia and give 28 to 50 μ as the range in size; the size recorded by Spegazzini is 50 to 80 μ and ours 45 to 75 μ.

In determining the identity of the downy mildew of cultivated tobacco, consideration must also be given to the fact of its ability to parasitize numerous species of Nicotiana, including *N. longiflora*, and certain other solanaceous species. This points to the conclusion that it is identical with *P. nicotianae* or else that there are 2 species of Peronspora on Nicotiana. In the case of the latter alternative, the downy mildew of cultivated tobacco is to be regarded as an undescribed species. It seems preferable, however, in the light of the facts that have been recounted, to regard the pathogen on cultivated tobacco as identical with *P. nicotianae*.

### PATHOLOGY OF DOWNY MILDEW

In studying the relation of the organism to invaded tissues and to the changes that the fungus produces in them use was made of stained, paraffin

sections and of preparations made by stripping off the epidermis. Penetration is accomplished by entrance of the infection hypha through the stomates, (Fig. 3, D), as reported by Darnell-Smith (8). The coarse, coenocytic mycelium that arises passes between the cells and, thence, throughout the leaf tissue. Branched haustoria, (Fig. 3, J), are formed, which penetrate the cell walls. This is best demonstrated in epidermal strippings. Four to 7 days after inoculation, sporangiophores are produced. With the production of sporangia the cells die and rapidly collapse. This collapse is not confined to the tissues actually invaded but may involve the entire leaf or entire plants. This effect on uninvaded tissues is borne out by the experience of many growers who transplanted diseased seedlings or apparently quite normal plants from seed beds in which the disease was rapidly developing. When this was done, both in 1932 and 1933, usually 50 to 90 per cent of the seedlings failed to survive transplantation unless transplanting was delayed until the seedlings had apparently recovered. It appeared that the actual loss of leaf surface, resultant from leaf lesions was insufficient to account for this high mortality in transplanting diseased seedlings. For this reason attempts were made to determine if toxic products are produced within diseased tissues. Accordingly, a quantity of fresh leaves of diseased seedlings were macerated by grinding in a meat chopper. The crushed material was then covered with distilled water and allowed to extract for 15 hours at 38° F. The liquid portion was then removed by filtration through filter paper and placed in 5 small flasks. Four healthy tobacco seedlings were then removed intact from the soil and placed, with their root systems immersed, in the filtrate. The filtrate in the fifth flask was heated to boiling before placing a seedling in it. A filtrate from healthy tobacco seedlings was similarly prepared and 2 seedlings were placed in it to serve as a control. After 20 hours the 5 plants in the extract from seedlings affected with downy mildew were severely wilted, whereas those in the filtrate from healthy seedlings were turgid. The roots of the wilted plants were then rinsed and allowed to remain in tap water to permit recovery of turgidity. No recovery resulted, however.

In a comparable test, dead dried leaves from diseased seedlings, instead of fresh ones, were extracted in distilled water over night, at 38° F. This extract also caused healthy seedlings, whose roots were immersed in it, to wither. An extract, similarly prepared, from apparently normal leaves of seedlings just beginning to recover from downy mildew, also caused healthy seedlings to wither when their roots were immersed in it. In this case, however, the wilting was less pronounced and was most noticeable on the lower leaves. These tests are interpreted to show that toxic water-soluble substances are formed within diseased tissues and that they may permeate

the entire plant. These toxic substances may account, in part, for the death of affected seedlings following transplantation.

### DISSEMINATION OF SPORANGIA

There are several agencies concerned in the dissemination of sporangia of downy mildew of tobacco. The spread of the disease either within an affected seed bed or from a diseased seed bed to a near-by healthy one could be readily accomplished by carriage of sporangia on the hands and clothing of laborers, (2), (13). It also has been demonstrated that, in Australia, sporangia are transported by moths of the split form, *Phthorimaea operculella* Z. (5).

The fact that the sporangia are powdery and so readily dislodged suggests that air currents should constitute the most important agency for their dissemination, as indicated in previous investigations (2), (13). Direct evidence of the transport of sporangia by air currents was obtained by means of spore-traps. These traps consisted of glass plates, 3¼ by 4¼ inches, covered with a film of glycerine and exposed, usually to leeward, in the vicinity of diseased seed beds. After exposure, the film of glycerine and adhering material were washed off, the washings were then centrifuged, and the sediment was examined with a microscope.

It was also found that sporangia are entrapped by glandular hairs on tobacco leaves, on the corollas of *Azalea nudiflora* or on the leaves of *Liquidambar styraciflua,* and by the resinous young shoots of pines. They could be washed off from these plant materials and collected in the sediment in centrifuge tubes. Some difficulty was experienced in identifying the sporangia of *Peronospora nicotianae* because 3 other morphologically distinct downy mildews were more or less prevalent; namely, *P. parasitica* (Pers.) deBy., on cruciferous weeds, *P. viciae* (Berk.) deBy., on *Vicia angustifolia,* (L.) Reichard, and *P. geranii* Pk., on *Geranium carolinianum* L.

The results of studies on trapping sporangia of the downy mildew of tobacco are summarized in table 1.

It may be noted, first of all, that sporangia occurred on most of the glycerine slides and on most of the plant material. In some cases a single sporangium or at most a few were found in the sediment; in others sporangia were abundant. It is conceivable that the number of sporangia in the air would be governed by such factors as distance from infected beds and the wind velocity. Diseased beds from which these sporangia were liberated varied from a few feet to a few miles. The wind velocity at the time when the sporangia were entrapped varied from 200 to 400 feet per minute.

When it was determined that sporangia are air-borne, an effort was made to utilize this fact in predicting the occurrence of an outbreak at a

TABLE 1.—*Dissemination of downy mildew by air currents*

| Date | Locality | Material examined | Results |
|---|---|---|---|
| 4–13–33 | Wilson, N. C. | Glycerine slide exposed at edge of diseased seed bed | Sporangia found |
| " | " | Glycerine slide exposed 25 ft. from diseased bed | No sporangia found |
| " | " | Glycerine slide exposed inside diseased bed | Sporangia found |
| " | " | Leaves of tobacco seedlings from bed 1000 ft. distant from diseased bed | " " |
| 4–14–33 | " | Leaves of *Liquidambar styraciflua* near diseased bed | " " |
| " | " | Glycerine slide exposed 1000 ft. distant from diseased bed | No sporangia found |
| 4–15–33 | " | Leaves of tobacco collected 1000 feet distant from diseased bed | Sporangia found |
| " | " | Flowers of *Azalea nudiflora* 300 ft. distant from diseased bed | |
| " | " | New shoot of pine collected near diseased bed | " " |
| 4–17–33 | " | Leaves of tobacco seedling collected at 8: 30 A. M. 6 hrs. after rain, at a point 50 ft. distant from diseased bed | " " |
| " | " | Leaves of tobacco from healthy bed 1000 ft. distant from diseased bed | " " |
| " | " | Flowers of *Azalea nudiflora* near diseased bed | " " |
| " | " | Glycerine slides (5) exposed 20, 100, 225, 300 and 600 ft. to leeward of diseased bed | " " |
| 4–23–33 | " | Tobacco leaves from plant bed that showed no evidence of infection | No sporangia found |
| " | " | Tobacco leaves from plant bed that showed no evidence of infection | " " " |
| " | " | Glycerine plate exposed for a few minutes near slightly infected bed | " " " |
| " | " | Glycerine plate exposed 2.5 hrs. near apparently healthy bed located 300 ft. from bed in which fungus was sporulating freely | Sporangia found |

TABLE 1.—(*Continued*)

| Date | Locality | Material examined | Results |
|---|---|---|---|
| 4–23–33 | Wilson, N. C. | Tobacco leaves from apparently healthy bed | Sporangia found |
| " | " | Tobacco leaves from apparently noninfected bed 4 miles west of Wilson | " " |
| " | Bailey, N. C. | Tobacco leaves from apparently healthy bed | " " |
| " | " | Young pine branch collected between Bailey and Middlesex | " " |
| " | Middlesex, N. C. | Young pine branch. Few diseased plants in bed 90 ft. distant | " " |
| " | Wakefield, N. C. | Young pine branch. Diseased bed 1 mile distant | " " |
| " | " | Young pine branch. Mildew just appearing in an occasional bed in this district | No sporangia found |
| " | Durham, N. C. | Glycerine plate exposed 24 hrs. 7 miles east of Durham between Durham and Wake Forest | " " " |

point distant from diseased beds. The situation chosen for this purpose was the Tobacco Experiment Station, Oxford, N. C. There were 2 foci of infection about 10 to 12 miles distant, one southeast and the other southwest of Oxford. Glycerine plates were exposed for 24 hour periods for 11 days prior to the outbreak of the disease. Seedlings were collected daily and their leaves examined for the presence of sporangia. The data for this series of examinations are assembled in table 2.

It will be seen that sporangia were first trapped at Oxford, N. C., on April 25. A period of weather that was continuously very favorable for the development of sporangia and their spread occurred from April 19 to April 22. The first evidence of infection in the seed beds at Oxford was noted on May 2, making an interval of 7 days between the date when sporangia were first found and that when a new crop of sporangia had formed. It should be recalled that this interval corresponds with the cycle of development of the disease.

### SOURCES OF INOCULUM IN SPRING

In previous investigations, three sources of inoculum for the initiation of the disease in spring have been suggested: (1) old tobacco plants that survived the winter, (2) infected seed, and (3) infested soil.

In parts of Australia (2) many plants survive the winter and, in spring, produce suckers upon which downy mildew lesions appear. While all

TABLE 2.—*Dissemination of sporangia by air currents, and its relation to the development of downy mildew at Oxford, N. C.*

| Date | Material examined | Place collected | Results |
|---|---|---|---|
| 4–21–33 | Tobacco leaves | Seed bed on new site | No sporangia found |
| " | " " | " " " old " | " " " |
| 4–22–23 | Glycerine plates (3) | North, south and east of seed beds | " " " |
| " | Tobacco leaves | Seed bed on new site | " " " |
| " | " " | " " " old " | " " " |
| 4–24–33 | Glycerine plates (3) | North and south of seed beds | " " " |
| 4–23–33 | Tobacco leaves | Seed bed on new site | " " " |
| " | " " | " " " old " | " " " |
| 4–25–33 | Glycerine plates (4) | North, south and east of seed beds | Sporangia found |
| " | Tobacco leaves | Seed bed on new site | " " |
| " | Glycerine plates (4) | North, south and east of seed beds | No sporangia found |
| " | Tobacco leaves | Seed bed on old site | Sporangia found |
| 4–26–33 | " " | " " " new " | " " |
| " | " " | " " " old " | " " |
| 4–27–33 | " " | " " " new " | " " |
| " | " " | " " " old " | " " |
| 4–28–33 | " " | " " " new " | " " |
| " | " " | " " " old " | " " |
| 4–30–33 | " " | " " " new " | " " |
| " | " " | " " " old " | " " |
| " | Glycerine plates (3) | North, south and east of seed beds | " " |
| " | " " (3) | North, south and east of seed beds | " " |
| " | Flowers of *Azalea nudiflora* | Within 5 ft. of seed bed | " " |

aboveground parts of tobacco plants are killed by frosts, except in occasional years, throughout the flue-cured tobacco area, it is of common occurrence for suckers to arise in spring from the underground portions of stubbles that have remained alive. Because of the mild winter, plants remained

alive in eastern North Carolina until March, 1932. Such plants therefore may have served as sources of inoculum for the wide-spread, early infections in seed beds that season.

Angell (1) was led to conclude that infected seed is an important source for the initiation of the disease in seed beds in Australia because of the occurrence of lesions on capsules, the presence of coenocytic mycelium in the seed from such capsules, the early appearance of the disease on seedlings grown from infected seed, and the occurrence of mildew on tobacco 20 miles away from the nearest plantation. No evidence has yet been found that such is the case within the United States.

During a two-week period in late March and early April, 1933, isolated outbreaks of the disease occurred throughout North Carolina in the localities indicated in figure 4. Doubtless numerous other outbreaks occurred to which our attention was not directed. These early outbreaks, that occurred a month or more prior to the season for transplanting, may be noted to be distributed rather generally throughout the tobacco-growing section. Investigation of these outbreaks established the fact that the seed beds in which downy mildew first appeared were located on or near sites occupied the preceding season by infected beds. This suggests the possibility that downy mildew may have survived the winter in the débris remaining from diseased seedlings grown in 1932. This assumption is strengthened by the facts that the pathogen forms oospores within the tissues of decaying leaves and that oospores of downy mildews generally, are known to constitute the hibernating stage.

Since it has not been possible thus far to germinate the oospores of the tobacco downy mildew, no definite knowledge is available regarding their period of dormancy, the conditions for their germination, and their relation to the initiation of the disease on seedlings. It is believed, however, that the fungus hibernates as oospores in occasional old seed beds and that oospores are the most important source of inoculum for the initiation of the disease in spring. Evidence in support of this belief is based (1) on the observation that the earliest occurrence of the disease, in 1933, was in beds located on sites utilized for seed beds during 1932; and (2) on the results of surveys to determine the extent and rate of spread of the disease from these beds. Surveys were made of areas surrounding foci of primary infections in three cases. It should be recalled that sporangia have previously been shown to be air-borne. The results of each survey were similar to the one shown in figure 5, made in Granville County. In this case the disease remained confined to the old bed, in which it first appeared about April 7, until the last few days of that month. On April 24 there was no evidence of downy mildew in any other seed beds in the area shown in figure 5. The period from April 19 to 22 was, however, very favorable

for sporulation. When this area was next surveyed, on April 30, the disease had attacked all the plants in a bed about 50 yards distant from the old bed that served as the primary center, and it was just beginning to make its appearance in the others indicated on the map. Most of these seed

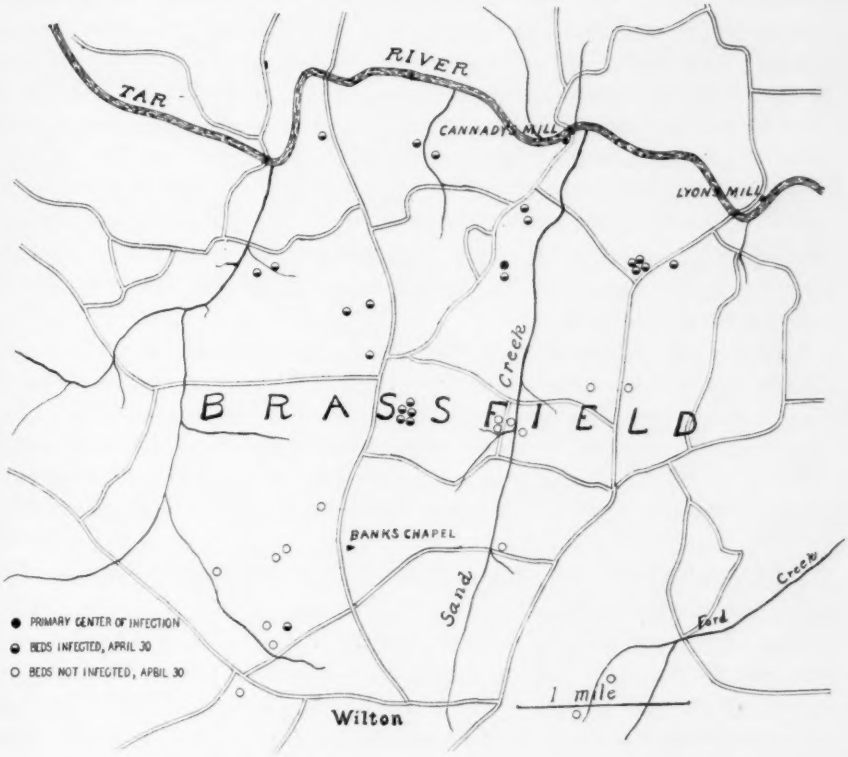


FIG. 5. A portion of Brassfield township, and adjacent territory in Granville County, N. C., showing the locus of a primary center of infection. The position of other seed beds in which infection was evident on April 30 is also indicated.

beds were in a northwesterly direction from the primary center. No evidence of infection was found in seed beds situated north of the Tar River. Two days later, however, the disease was beginning to appear, not only throughout this entire area, but was scatteringly present in seed beds 10 to 15 miles distant.

## GERMINATION OF SPORANGIA

The sporangia of *Peronospora nicotianae* are extremely variable in their capacity for germination. Among the factors that influence germination are moisture, temperature, light, and reaction of the medium. Germination

cannot take place except in a film of water or in a saturated atmosphere. If sporangia are powdered onto a slide and placed in a container with a relative humidity of 95 per cent, they will not germinate. Ordinarily, when freshly formed sporangia are placed in drops of water on microscope slides, and are maintained at about 70° F. from 50 to 90 per cent will germinate within 5 hours. A single, lateral, crooked or tortuous germ tube usually is formed, (Fig. 3, B), whose growth soon ceases. At 45 to 60° F. germination is accomplished within 2 hours. Sporangia maintained for 22 hours at 79° F. had not germinated, but a portion of them were found to be still viable. When exposed for 8 hours at 82° F. the sporangia were still capable of germination, but were killed by exposure to this temperature for 42 hours. When freshly formed sporangia were powdered onto dry slides, however, and exposed, in darkness, to a temperature of 84° F. for 1 hour they were found incapable of germination when placed under conditions favorable for germination.

Sporangia germinate as readily in diffuse light as in darkness, as shown in a series of trials. When, however, sporangia are kept continuously in drops of water in a container placed on a block of ice and are exposed to direct sunlight, they are incapable of germination after an hour's exposure. Angell and Hill (2) found that sporangia lost their viability in less than an hour's exposure to direct sunlight, at a time when the shade temperature was 23° C.

Malic acid and bicarbonate of soda in aqueous solution were used in testing the effect of reaction of the medium on germination of sporangia. A series of solutions whose pH concentration, as determined electrometrically, ranged from pH 3.63 and pH 8.8 were employed. The sporangia germinated in each of the different concentrations, but germination was most vigorous and appeared to be stimulated throughout the acid range. In the alkaline range the germ tubes were short and the percentage of germination was much reduced.

### VIABILITY OF SPORANGIA

Consideration was given to some of the factors that conceivably influence the length of time that sporangia of *Peronospora nicotianae* remain viable, because of the fact that they are subjected to varying environmental conditions between the time that they are mature and find lodgment after having been carried through the air. Seed beds in which they lodge are, in certain instances, known to be some miles distant. The factors concerned include temperature, light, and age of the sporangia.

In general, the sporangia are short-lived, approximately one per cent only being viable after 4 to 7 days. Angell and Hill (2) report as an extreme case the viability of sporangia 117 days old, after they had been kept

in air-dry soil at a temperature of 3° to 5° C. Differences in relative humidity appear to be of no significance in modifying viability. A series of relative humidities ranging from 10 per cent to 95 per cent, were maintained in desiccators over different percentages of sulphuric acid. As a result of several series of trials maintained at a temperature of 76° F., it is concluded that sporangia are equally viable after exposure to any humidity within this range. If sporangia are subjected to a temperature of 79° F. under conditions of saturation they begin to lose their viability after about 21 to 24 hours. With longer exposures, there is a rapid increase in the death rate. With lower temperatures there is an increase in the length of time that sporangia remain viable, indicating that longevity is correlated with low temperature.

Angell and Hill (4) found that sporangia stored under moist conditions, at 18° C., remain viable for 2 months, and when stored over fused calcium chloride at this temperature (3), for 5 weeks.

As previously shown, direct sunlight is lethal to sporangia exposed for an hour. When sporangia from the same source as those exposed to direct sunlight are stored at a constant temperature and are exposed to diffuse light they remain viable for several days.

## RELATION OF METEOROLOGICAL CONDITIONS TO THE OCCURRENCE OF DOWNY MILDEW

Unsuccessful attempts were made to study the course of the disease under conditions of constant temperature, 76° F., and at each of the following relative humidities: 55, 65, 75, 85, 95, and 100 per cent. Potted tobacco plants were inoculated by wetting the leaves with a suspension of sporangia. They were then sealed under bell jars and a steam of air, conditioned by being bubbled through appropriate concentrations of sulphuric acid, was continuously passed through these jars. By adjustment of the rate of flow of the conditioned air, fairly constant relative humidities were maintained, as shown by psychrometers suspended inside the bell jars. If the films of moisture were permitted to remain until infection had been accomplished, lesions developed. No fructifications of the pathogen appeared, which was doubtless due to the high temperature.

Observations indicate that the course of the downy mildew is closely circumscribed by weather conditions. This fact might be anticipated from the results of studies previously recounted on the effects of environmental factors on germination and viability of sporangia. Additional evidence bearing on weather as a modifying factor was obtained by means of a Friez hygrothermograph. This instrument was placed within a tin box with numerous perforations in the sides and ends to permit easy air circulation. A cloth or a sheet of Celotex was placed on top of the tin box to prevent

heating by direct radiation. This box was placed upon the ground within the seed bed. In addition, records were made of the character of the sky and of rainfall.

During a 7-day period, beginning April 15, the instrument was placed in an apparently healthy bed, that was located about 25 yards away from a bed that was first noticeably infected about March 30. Several small spots developed during the week in which the instrument was in this bed. In the following week, beginning April 23, the hygrothermograph was

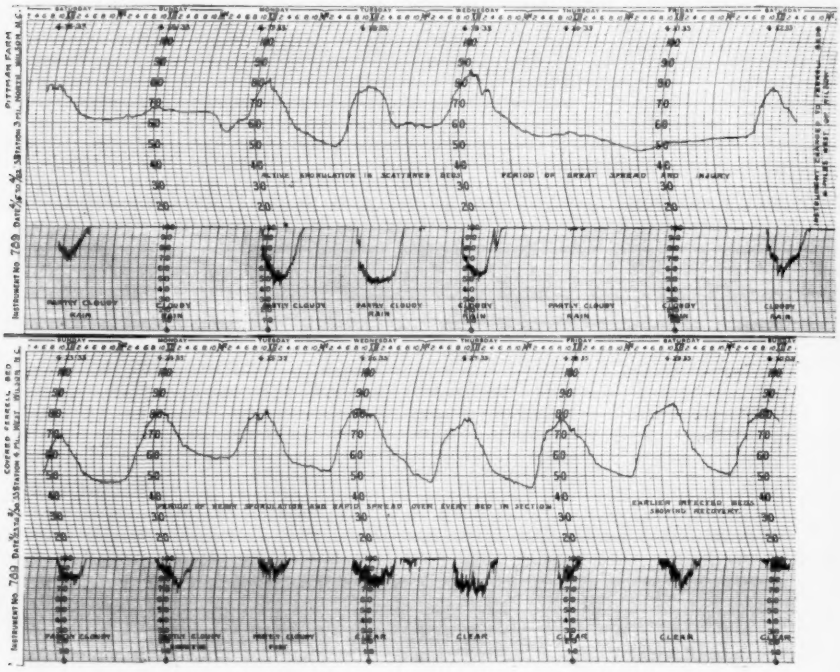

FIG. 6. Meteorological record for near Wilson, N. C., for the period from April 15 to April 30, 1933. The hygrothermograph was placed within the seed beds indicated.

placed in another bed about 5 miles distant. Examination of the leaves of the seedlings in this bed showed that sporangia were present, but there was no evidence of infection. Infection was first apparent the preceding day in a bed about 100 yards distant from that in which the instrument was placed. In this case downy mildew developed throughout the entire bed during the week in which the instrument was located in it.

The meteorological record for this 2-week period is shown in figure 6.

Attention should be called to the period from the evening of April 19 to the morning of April 22, when the temperature remained below 60° F.,

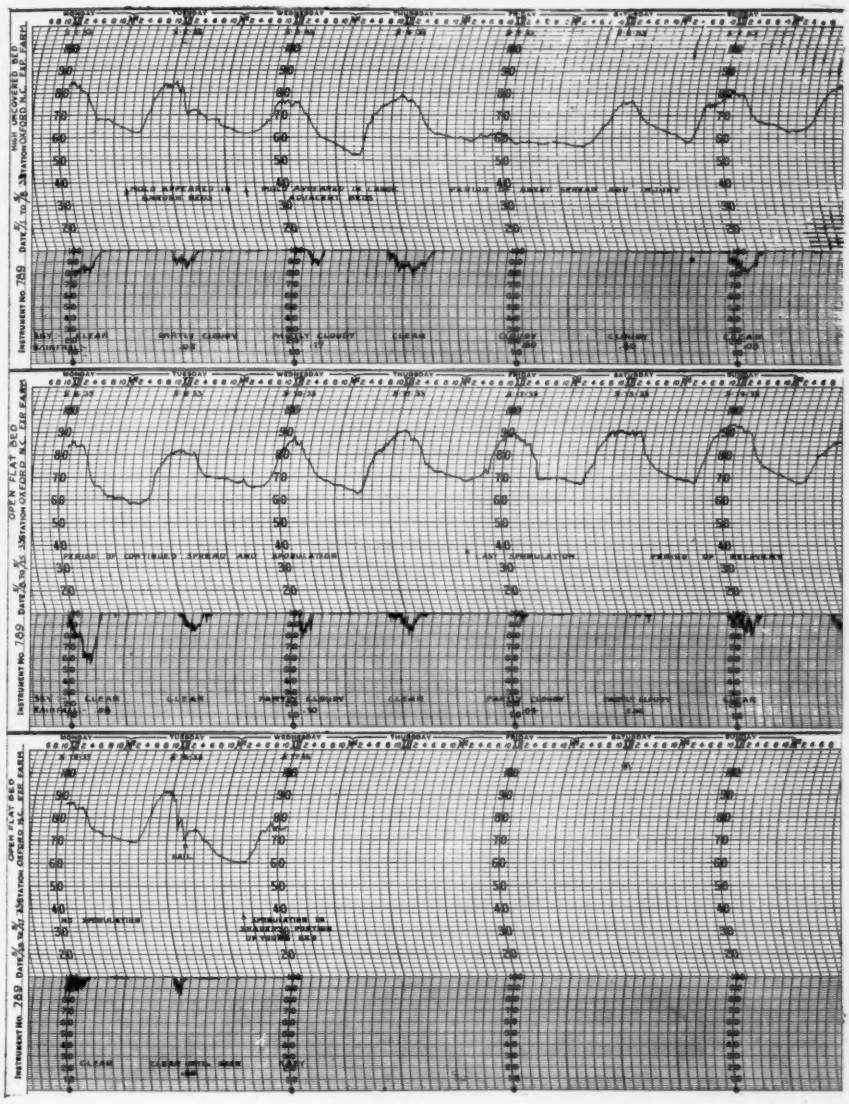

FIG. 7. Meteorological record for the Tobacco Experiment Station, Oxford, N. C., for the period from May 1 to May 17, 1933.

the sky was almost continuously overcast and the relative humidity remained at the point of saturation. These conditions are optimum for sporulation, dissemination, and infection; and, in consequence, the disease appeared in every bed in the section during the fore part of the following

week. It may also be noted that temperature and humidity conditions favored sporangial production every night during the week beginning April 23. The 5 successive, clear days of the last of the week with maximum temperatures of approximately 80° F. were favorable, however, for the rapid growth of infected plants.

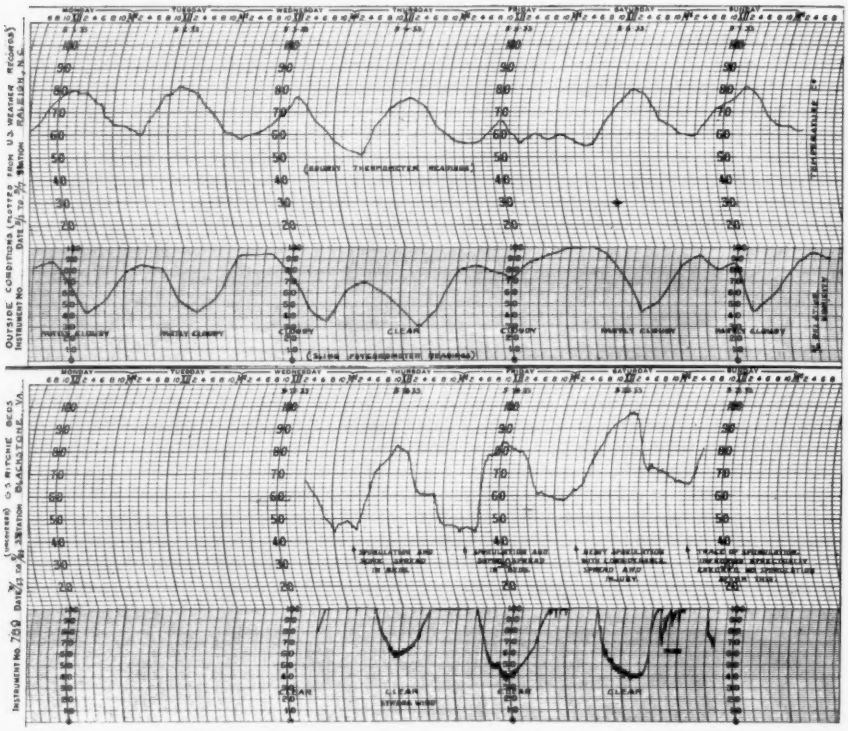

FIG. 8. Meteorological records. The upper graph is taken from data collected at the U. S. Weather Bureau Station, Raleigh, N. C. Compare it with the corresponding period in figure 7. The lower is a hygrothermographic record made within a seed bed at Blackstone, Va., May 17 to 21, 1933.

The hygrothermograph was placed in a seed bed at the Tobacco Experiment Station, Oxford, N. C., for the period from May 1 to 17, inclusive. The meteorological data given in figure 7 show the conditions that prevailed from the first appearance of downy mildew throughout the course of the disease and extending to the eventual recovery of the seedlings.

It may be noted that infection was first evident in some experimental seed beds in the garden in the morning of May 2. On the following morning it made its appearance in the seed beds, located about a half mile distant, from which the crop was planted. The disease did not assume

epiphytotic proportions until the period extending from the evening of May 4 to the morning of May 7, when the temperature almost continuously approximated 60° F. and the relative humidity remained at the point of saturation. This "downy mildew" weather was followed by a week favorable for the growth of tobacco, but with day temperatures between 80 and 90° F. that were unfavorable for the pathogen. The first three nights of this week were quite favorable for sporulation but only a slight amount of sporulation occurred subsequently. The period from April 11 to 17 was one in which temperature and rainfall favored the rapid growth and recovery of the seedlings. Hail, which fell during the afternoon of April 16, lowered the night temperature sufficiently so that sporulation again occurred in a near-by, late-sown bed that contained very young seedlings. The temperature of the 2 following days remained below 80° F., the temperature at night fell to between 50 and 60° F., and there was abundant production of sporangia on the young plants.

The temperature and humidity conditions recorded by the U. S. Weather Bureau Station at Raleigh, N. C.,[4] are not taken at the surface of the soil and should not be expected to correspond with those shown by the Friez hygrothermograph placed within seed beds. These differences are typified by comparison of the record for May 1 to 7, shown in figure 8 with that of the corresponding period at Oxford, N. C., about 40 miles distant from Raleigh, shown in figure 7.

The temperatures shown by the graph in figure 8 were plotted from hourly readings and the humidity graph from sling-psychrometer readings made at intervals of 4 hours; namely, at 4 and 8 in the forenoon, at noon, at 4 and 8 in the afternoon, and at midnight. The temperature graphs for these two places correspond more nearly than do the humidity graphs. The discrepancies in humidity can be explained if it is recalled that dew usually collects in tobacco seed beds at sunset and the seedlings remain covered with drops of dew all night long. Droplets of water remain on the lower leaf surface, moreover, for the greater portion of the day, unless the wind is blowing rather vigorously.

Downy mildew first appeared at Blackstone, Va., about May 14. On the nights of May 17, 18, and 19 (Fig. 8), meteorological conditions were ideal for sporulation. On the 20th the seed beds had the appearance of having been scalded. Temperatures in excess of 90° F., which prevailed that day between 11:00 A. M. and 5:00 P. M., together with unobstructed sunshine, effectively checked the further spread of the disease. A meager crop of sporangia were formed on the night of April 20. Subsequent observations showed that in the vicinity of Blackstone, Va., the disease disappeared al-

[4] The complete weather records for March, April, and May, 1933, were made available for study through the courtesy of Mr. L. A. Denson.

most as suddenly as it had come, and after 2 or 3 additional hot days those tobacco seedlings that survived were well on the way to recovery.

The lethal effects on sporangia of temperatures of 84° F. and above and of direct sunlight, as previously shown, account for the disappearance of mildew with the advent of summer weather. Although the organism had almost disappeared from the vicinity of Durham, N. C., by May 15, it was kept alive under "artificial" conditions, with the production of a crop of sporangia as late as July 11. This was accomplished by digging a pit at the edge of the woods, putting blocks of ice into the pit, and then placing potted inoculated plants directly upon the ice. By transferring fresh sporangia as they appeared, to healthy tobacco seedlings, and returning them to this "refrigerator," the pathogen has been made to pass through its pathogenic cycle repeatedly, even though the maximum daily temperature was 85° F., or above for 42 of the 56 days from May 15 to July 11, and 90° F. or above for 29 days of this period.

## PREVENTION AND CONTROL

Satisfactory prevention and control of downy mildew of tobacco seedlings has not been accomplished. Nevertheless, it is indicated from the investigations that have been made and from observations that certain practices should have a palliative influence. The fact that the disease appeared in seed beds of all types of construction now employed suggests the need for experimentation on this phase of the problem. Tightly constructed seed beds with covers of closely woven texture stimulate rapid growth of seedlings, and interfere with proper ventilation. Plants grown in such beds are apparently most liable to severe injury. In order to facilitate ventilation, the employment of narrow beds and the early removal of the frames is indicated. The slight elevation of such beds above the level of the surrounding soil should also be beneficial.

Sites on high ground are to be preferred to low lying, swampy situations; sites in open fields, to those closely surrounded by woods; and beds on steep slopes, exposed to good opportunities for air drainage, to those on level situations. More severe injury occurs in seed beds that are shaded during a portion of the day than in those more distant from the woods.

Thinly sown beds and those containing less than half of the usual stand of seedlings suffer less damage than thickly sown beds.

It is customary to remove the seed-bed covers a few days or at most a week prior to the time for transplanting. When the covers are removed 3 or 4 weeks before the season for transplanting and are returned at night only if frost is indicated, the seedlings are more hardy and less subject to serious damage.

The influence of temperature on sporulation and on viability of spo-

rangia indicate experimentation on artificially heated beds. This is also indicated by the necessity of moisture for germination and infection. Angell and Hill (2) have suggested the use of seed beds so constructed as to make possible the control of temperature and humidity.

Seed beds should be made in new situations each year. The use of sites occupied by beds during the previous year apparently constitutes the most important source of primary infection. This observation is probably the most significant one in the present investigation. Beds on old sites may be expected to become infected about 3 or 4 weeks prior to the general prevalence of downy mildew. Such beds are continuous sources of sporangia that are spread by air currents to the nearest seed beds and thence to more distant ones until eventually all seed beds are infected. Universal avoidance of old bed sites may be expected to be an effective preventive procedure.

The destruction of all tobacco seedlings and débris that remain in the seed bed, as soon as the crop is transplanted, is indicated by the fact that *Peronospora nicotianae* forms oospores.

When the disease appears, applications of nitrate of soda should be made to stimulate the recovery of diseased seedlings.

Transplanting seedlings from infected beds is counter-indicated. Instead, they should remain undisturbed until after recovery is advanced to the stage that new leaves and new roots have formed.

## SUMMARY

Downy mildew or blue mould of tobacco has for years been seriously destructive to the crop, especially to seedlings in Australia. In 1931 it appeared in the Florida-Georgia district in epiphytotic proportions. It recurred in the two subsequent years and spread so that it now is ubiquitously present throughout the southeastern United States in the area devoted to the culture of flue-cured tobacco. It has also extended its range into the Burley tobacco-growing sections of Tennessee and western North Carolina and into Maryland and Pennsylvania.

Many species of Nicotiana and many agronomic varieties of cultivated tobacco are subject to attack. It is pathogenic, in addition, to seedlings of tomato, pepper, and eggplant.

Downy mildew of tobacco is a necrotic disease. Small seedlings succumb to its attack; many older ones survive. Fructifications of the pathogen form a copious downy covering over the lower leaf surface, the most characteristic sign by which the disease may be identified.

The disease is caused by *Peronospora nicotianae* Speg. Its pathogenic cycle covers from 4 to 7 days. Sporangia are produced in the interval between daybreak and sunrise, and appear a few hours before death and col-

lapse of the invaded tissue. Oospores form within the dead tissues and are mature 4 to 7 days after death of the cells. Nothing is known of their germination.

The germ tubes enter by way of the stomata, and the mycelium is intercellular. Digitate haustoria penetrate the cells. Toxic water-soluble substances are formed within diseased tissues and their presence accounts, in part, for the high mortality rate when diseased seedlings are transplanted.

Sporangia have been trapped at long distances from diseased seed beds, which shows that they are air-borne. By means of spore-traps, sporangia were found at one location a week prior to an outbreak of downy mildew.

Seed beds situated on or near the sites occupied by old seed beds have been found to constitute primary centers of infection in early spring.

Moisture is necessary for germination of the sporangia of *Peronospora nicotianae*. Exposure for about an hour to direct sunlight is lethal. Germinations have not been accomplished at 79° F. or above. Continuous exposure of sporangia to a temperature of 82° F. and exposure for an hour at 84° F. destroys their viability. Sporangia are relatively short lived, except at low temperature, and viability does not appear to be modified by storage at different relative humidities.

Meteorological conditions exert a profound influence upon the course of the disease. The disease is checked by clear days with temperatures of 84° F. and above. Rainy weather, with continuously overcast skies and temperatures of 50 to 60° F., is ideal for sporulation and for the dissemination and germination of sporangia. *Peronospora nicotianae* has been made to repeat its pathogenic cycle under artificial environmental conditions.

Methods of prevention and control have not been determined. The avoidance of placing seed beds on sites utilized for seed beds during the previous year should be of primary importance, if universally practiced. It is recommended also that plants and débris remaining in seed beds after the crop has been transplanted be destroyed. The beds should be thinly sown, should be situated to provide ample air and soil drainage, and the covers should be removed early to render the seedlings more hardy.

When downy mildew appears applications of nitrate of soda should be made to stimulate the recovery of the seedlings.

Transplanting should be delayed until the plants have recovered, since a large proportion of the seedlings perish if transplanted from seed beds in which the disease is actively developing.

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# TEMPERATURE STUDIES ON STRIPE OF BARLEY[1]

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## INTRODUCTION

Although stripe of barley caused by *Helminthosporium gramineum* Rabh. has been recognized and studied for many years, it was not until relatively recently that the environmental conditions affecting the development of this disease have received major attention. The development of suitable inoculation technique has made possible a more critical study of the environmental factors influencing stripe. Because spore production in culture on ordinary media has not been obtained, any method of inoculation involving conidia has depended upon infected plants. Natural infection during the early stages of kernel development has not been consistently high enough to depend upon this method for infected seed. Therefore, most of the earlier workers were dealing with infection percentages too low to make their results especially significant. Although mycelial production in culture has been abundant, difficulties in using this source of inoculum in seedling infection were not overcome until relatively recently.

The influence of temperature upon stripe development of barley has been discussed by various workers, beginning with Ravn (10). Several of the investigators have attempted to give a rather definite optimum temperature for disease production, varying from 0–8° C. by Isenbeck (6), to 15° C. by Leukel, Dickson, and Johnson (8). The differences in reported optimum temperatures may be accounted for in part by different technique, host varieties, and cultures of the parasite used in the several investigations. However, as stated earlier, the relatively low stripe infection obtained by the earlier workers has made much of the early temperature studies difficult to interpret. The results here presented would indicate that it was impossible to specify any condition as optimum unless such modifying factors as method of inoculation, host variety, culture of the parasite, etc. were defined specifically in relation to the variable environmental factor under consideration.

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[2] The writer wishes to make grateful acknowledgement to Dr. J. G. Dickson for advice given during the course of study, and for aid in the preparation of the manuscript. He also wishes to thank Prof. B. D. Leith for counsel during the investigation, Miss Helen Johann for criticism of the manuscript, and Mr. Eugene Herrling for aid in the preparation of the illustrative material.

## RELATION OF TEMPERATURE TO GROWTH OF HELMINTHOSPORIUM GRAMINEUM IN CULTURE

Five strains of *Helminthosporium gramineum* selected from widely different geographical regions were grown in 2 series on potato-dextrose agar at constant temperatures from 8° to 32° C. arranged at 4-degree intervals. The 5 cultures were selected on the basis of differences in pathogenicity and apparent differences in temperature relations with reference to disease production. The cultures used were: (1) 21a1, a monoconidial culture, isolated at Madison, Wisconsin; (2) 41, a culture of monoconidial origin from Lincoln, Nebraska; (3) Selo 2, a culture of monoconidial origin from Detskjoe Selo, near Leningrad, Russia; (4) Ariz. 1, a mycelial isolation from Arizona; and (5) Ind. 1, also a culture of mycelial isolation from La Fayette, Indiana. Approximately equal bits of mycelium of the same age were transferred to each Petri dish; the inoculated dishes were then placed immediately in chambers operating at the temperatures desired. Two series of 5 plates each were employed in determining the average colony diameter. The diameter of each colony was measured at the end of 6½ days after inoculation. The average diameter is recorded in table 1. Mycelial growth

TABLE 1.—*Effect of temperature on colony diameter of cultures of Helminthosporium gramineum when grown on unacidified potato-dextrose agar in Petri dish cultures. Diameter of colonies were determined 6½ days after starting. Average of 2 series of 5 plates each*

| Culture of fungus | Diameter in millimeters at temperatures given in degrees Centigrade | | | | | | |
|---|---|---|---|---|---|---|---|
| | 8 | 12 | 16 | 20 | 24 | 28 | 32 |
| 21a1 | 11.4 | 26.6 | 43.9 | 53.7 | 77.4 | 83.8 | 61.1 |
| 41 | 12.9 | 28.3 | 47.3 | 60.9 | 78.1 | 82.2 | 27.1 |
| Selo 2 | 7.0 | 15.3 | 34.0 | 34.6 | 51.7 | 51.2 | 19.0 |
| Ariz. 1 | 13.1 | 30.2 | 46.3 | 58.1 | 77.5 | 78.7 | 38.2 |
| Ind. 1 | 14.8 | 28.6 | 43.0 | 58.3 | 76.5 | 72.4 | 43.5 |

as measured by colony diameter was slow at 8°, increased rapidly with rising temperatures until 24° was reached, slowed up in growth rate with minor cultural changes at 28°, and dropped off markedly at 32° C. There was little difference in the temperature-growth response from 8° to 24° inclusive in the colonies of the cultures other than the Selo 2 isolation. At 28° the diameters of cultures 21a1, 41, and Ariz. 1 increased above those at 24°, while Ind. 1 and Selo 2 decreased slightly. The minimum temperature was considerably below 8° C., the lowest temperature used in the series. The optimum temperature on potato-dextrose agar was near 25° C. as Ravn

(10) reported when he used average daily increase in culture diameter on beer-wort agar for determining cardinal temperatures of growth. The maximum temperature was not quite reached at 32°, the highest temperature used in the study. Culture 21a1 seemed more tolerant of the higher temperature than any of the other isolations studied.

Modifications in the appearance of colonies, especially in the type of mycelium and in pigment formation were evident in addition to differences in diameter of the colonies at the various temperatures. The height and density of aerial mycelium in general, closely paralleled radial proportions, except the aerial mycelium of the Selo 2 isolation was scant at the lower temperatures, and Ariz. 1 was perhaps a little more dense, while the mycelium of 21a1 was tall and fluffy. In general, pigmentation of all cultures was least at the lower temperatures, and greatest at 32°. The different colonies showed considerable variation in intensity of color, and in zonation at the intermediate temperatures. Cultures 41 and Ariz. 1 formed pigment zones at 32 and 28°, and Ind. 1 formed growth zones at 16–28° inclusive. The shape of the colonies of 21a1, 41, and Ariz. 1 was almost circular at all temperatures, while Selo 2 and Ind. 1 were more irregular in circumference. The Selo strain produced the greatest variation in shape and general appearance. After 20 days' growth at 8°, colony diameters of cultures 21a1, 41, Selo 2, Ariz. 1, and Ind. 1 were 56, 59, 62.4, 68.8, and 72.8 mm. respectively. Culture 21a1 was the smallest at 8°, and largest at the higher temperatures.

### INFLUENCE OF TEMPERATURE ON THE DEVELOPMENT OF BARLEY

*Effect of Constant Temperature.* Although the study of the influence of a constant soil and air temperature upon the development of the barley plant has not been the main purpose of this investigation, a brief summary of the data taken upon the temperature responses of barley will aid in interpretation of the results obtained with reference to stripe expression after artificial inoculation. General notes were kept during the growth of several series, and specific data were taken on the uninoculated controls of one series after 40 days' growth.

Earliest germination of barley seedlings was favored by the temperatures of 28 and 32° C., where emergence took place in 3 to 4 days. The time of emergence was increased at 24, 20, 16, 12 and 8° C., respectively, 10–11 days being required for complete emergence at 8°. The growth and development of barley for the most part corresponded to that of spring wheat as described by Dickson (2). The coleoptile was longest at 16°, and shorter at both higher and lower temperatures. Likewise, the length of the subcrown internode was greatest at 16°, and shorter as the temperature increased or decreased. The diameter of the base of the culms was largest at

20°, slightly less at 16° than at 20°, and smallest at 32° C. The first leaf was shortest at 8°, and increased in length with rise in temperature to 24°, beyond which no data were taken. The first leaf was widest at 12°, and markedly decreased in width with temperature increase. However the later leaves were widest at 20°, with gradual decrease in width at higher and lower temperatures. The number of mature leaves and approximate height of tops, after 40 days' growth was correlated with rate of growth which reached a maximum at 24° C. The dry weight of tops was greatest at 24°, with a secondary maximum at 16° C. The general appearance of the barley plants after 40 days' growth at the several temperatures is shown in figure 1.

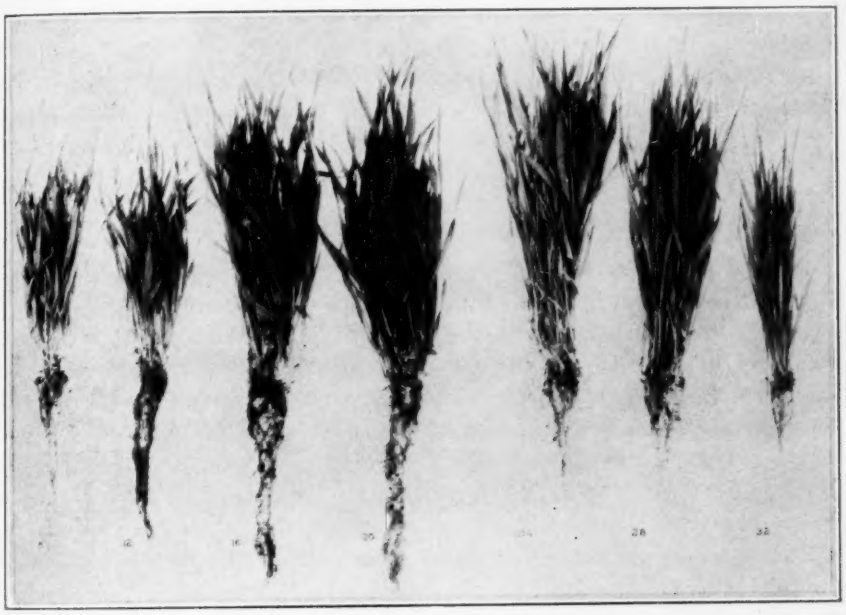


FIG. 1. Effect of soil and air temperatures on the development of Wis. Ped. 6–6 barley. Soil temperatures were maintained constant at temperatures indicated. Air temperatures were as follows: Plants at 8 and 12° soil temperature were grown in a greenhouse held at 8–12° C., 16° and 20° soil temperatures at 20° air temperature, 24° soil at 24° air, and 28 and 32° soil at 28° C. air temperature.

The length and dry weight of roots as compared with the tops of the various temperatures indicated a different temperature response for roots than for shoots of barley. The longest root growth was at 20°, with the heaviest dry weight at 12°, with only a slight decrease at 16° C. The largest total dry weight per plant, like dry weight of tops was produced at 24° where the tissues were relatively more mature than at the lower temperatures. Flow-

ering and maturation of grain in plants grown at the different temperatures progressed in the following order from first to last respectively: 24, 20, 16, 12, and 8° C. Abnormal flowering and very little grain production occurred at 28 and 32° C. As the seedlings increased in age, the temperature conducive to better top development gradually lowered. The greatest final development and yield of Wisconsin Pedigree 6-6 barley occurred at the lower temperatures 12–16° C., but the time required for completion of growth was likewise longer.

*Effect of Change in Temperature.* Change of temperature during the period of development of the barley plant had a marked effect on subsequent growth and appearance. When seed germinated and grew during the seedling stage at a low soil temperature and was later changed to a higher temperature the plants assumed a type of growth similar to that of plants that had grown continuously at the higher temperature. In like manner, when slender plants grown at a high temperature were transferred to a low temperature they soon developed a stocky growth not unlike those grown continuously at the lower temperature.

### RELATION OF TEMPERATURE TO STRIPE DEVELOPMENT

*Method of Inoculation.* Very little had been accomplished toward developing a method of inoculation that afforded stripe resembling that caused by natural floral infection until Johnson (7) began investigation of the possibility of infection resulting from seed or seedling inoculations. DeHaan (5), Genau (4), Winklemann (11), Fuchs (4), and Majdrakoff (9) tried several methods involving seed, seedling, and floral infection but were unable to produce consistently high percentages of stripe. Isenbeck (6) was the first to develop satisfactory methods for stripe inoculation. Flowers inoculated with dry conidia gave higher infection than when using conidial suspensions in water for floral inoculations. Isenbeck (6) also deglumed barley seed and, while still moist, rolled them in spore dust. This was the most severe type of inoculation used, giving higher percentages of infection than floral inoculations. When he placed deglumed seed in contact with chopped mycelium on agar, the results corresponded to those obtained with floral inoculations, but did not agree with the conidial inoculation of the seed. J. J. Christensen suggested that infection might be secured by placing barley kernels with the hull intact in contact with the fungus growing on sterilized wheat kernels.

In the present work, floral inoculations were carried out in the summer of 1930 by placing glassine bags over groups of plants just before the heads emerged from the uppermost leaf sheath. The heads were sprayed with a suspension of conidia obtained from diseased plants. More than 80 per cent stripe infection developed from seed florally inoculated in this manner

in 1930; but less infection was obtained by the same method during the less favorable seasons of 1931 and 1932.

Seed inoculations were made by placing the kernels between layers of potato-dextrose agar on which the fungus was growing, followed by an overnight incubation before planting. Seed in hull, acid-hulled, and hand-hulled kernels were inoculated in this manner, with the former giving relatively little infection, and the latter two methods resulting in much higher infection. The acid-hulled seed resulted in the highest per cent infection. The method of acid hulling modified from that of Briggs (1) was as follows: Barley kernels were stirred in concentrated sulphuric acid until the lemma and palea were digested, except for a portion over the embryo and a very thin layer over the remainder of the seed. The kernels were then quickly poured into a paraffin-coated wire basket, rinsed in cold water, and immersed in dilute alkali. The time required for the acid digestion of the hulls without injuring germination varied with the variety or selection of barley, and probably with the locality in which the seed was produced. The time required for acid digestion with samples of Wisconsin Pedigree 6-6 was 4 minutes for most of the experiments.

High percentages of seed infection were also obtained when kernels previously treated with 70 per cent alcohol for 15 minutes and rinsed in distilled water were inoculated by contact with cultures of the fungus growing on a wheat-kernel medium prepared by autoclaving equal parts by weight of wheat kernels and distilled water. This is essentially a modification of the method first suggested by Christensen in which he treated the barley kernels with an organic mercury dust prior to inoculation.

*Relation of Constant Temperature.* The influence of soil temperature on the development of stripe has been the subject of several investigations since the time of Ravn (10), who noted that more stripe occurred in early plantings of naturally infected seed than in later plantings from the same seed lot. Johnson (7) concluded that low temperatures favored infection, and that the critical period for infection was during, and immediately after seed germination. The total number of striped plants obtained was, however, so small as to make the data on temperature response inconclusive. Isenbeck (6) found relatively low temperatures, 0 to 8° C., and 25% soil moisture most favorable for infection and development of stripe. Leukel, Dickson, and Johnson (8) stated that the temperatures of 15° C. or below during the period of emergence favored stripe infection, and that temperatures above 20° C. inhibited the disease.

The temperature studies reported in this paper were conducted in the Wisconsin temperature tanks placed in greenhouses in which the air temperatures were controlled at 12, 16, 24, and 28° C. Soil composed of about 70–75% loam and 25–30% sand, with the moisture held within a range

TABLE 2.—*Influence of constant soil and air temperatures upon the development of stripe. Summary of experiments of 1931–1932*

| Temperature in °C. | | Determinations | Ped. 6-6 acid-hulled seed inoculated with culture | | C.I. 5024 hull-less seed inoculated with culture | Ped. 6-6 florally inoculated[c] | Ped. 6-6 acid-hulled seed uninoculated control[b] |
|---|---|---|---|---|---|---|---|
| Soil | Air | | 21a1[a] | 41[b] | 38[c] | | |
| 8 | 12 | Total No. | 152 | 127 | 86 | 63 | 60 |
| | | Striped No. | 71 | 78 | 36 | 8 | 0 |
| | | Striped per cent | 46.7 | 61.4 | 41.9 | 12.7 | 0 |
| 12 | 12 | Total No. | 191 | 139 | 86 | 64 | 75 |
| | | Striped No. | 84 | 69 | 67 | 9 | 1 |
| | | Striped per cent | 44.0 | 49.6 | 77.9 | 14.1 | 1.3 |
| 16 | 20 | Total No. | 225 | 152 | 82 | 66 | 93 |
| | | Striped No. | 104 | 76 | 35 | 18 | 0 |
| | | Striped per cent | 46.2 | 50.0 | 42.7 | 27.3 | 0 |
| 20 | 20 | Total No. | 229 | 155 | 86 | 61 | 71 |
| | | Striped No. | 120 | 85 | 32 | 19 | 0 |
| | | Striped per cent | 52.4 | 54.8 | 37.2 | 31.1 | 0 |
| 24 | 24 | Total No. | 191 | 144 | 78 | 62 | 67 |
| | | Striped No. | 104 | 80 | 23 | 15 | 1 |
| | | Striped per cent | 54.5 | 55.6 | 29.5 | 24.2 | 1.5 |
| 28 | 28 | Total No. | 219 | 142 | 83 | 61 | 88 |
| | | Striped No. | 81 | 49 | 8 | 1 | 0 |
| | | Striped per cent | 37.0 | 34.5 | 9.6 | 1.6 | 0 |

[a] Three series; [b] two series; [c] one series.

considered most favorable for plant growth, was used throughout the investigations. Three plantings of inoculated seed were made in the winter of 1931–1932 (Table 2). Wisconsin Pedigree 6–6 acid-hulled seed was inoculated with cultures 21a1 and 41, and C.I. 5024, a blue hull-less barley, was inoculated with culture 38, a monoconidial isolation from Wisconsin, by placing seed between layers of potato-dextrose agar on which the fungus was growing. The last named variety, C.I. 5024, and florally inoculated Pedigree 6-6 were included only in the third planting. The stripe resulting from the triplicate plantings of Pedigree 6-6 inoculated with isolation 21a1, and of the duplicate series of inoculations with culture 41 agreed as well as could be expected, and therefore are summarized in table 2. Pedigree 6-6 barley inoculated with cultures 21a1 and 41 resulted in 46.7 and 61.4% stripe respectively at 8° C., with a decrease at both 12 and 16°, and with an

increase at 20 and 24° C. The development of stripe in response to temperature was decidedly different when C.I. 5024 inoculated with culture 38, and 1931 Pedigree 6-6 florally inoculated seed were tested. The percentage of stripe obtained with C.I. 5024 was 77.9 at 12°, with much less both at 8° and at the higher temperatures. This barley variety showed a slight tendency to outgrow the stripe infection even though a lower leaf was distinctly infected. Florally infected Pedigree 6-6 showed a still different temperature reaction; the highest percentages of stripe developed at 20 and 16° C.

TABLE 3.—*Influence of constant soil and air temperatures upon the development of stripe. Summary of two series duplicated 1932–1933*

| Temperature in °C. | | Determinations | No. of plants used and stripe infection | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Ped. 6-6 inoculated with culture 21a1 | | C.I. 5024 hull-less inoculated with culture 38 | White hull-less inoculated with Ariz. 1 culture | ×76–2–6–1 florally inoculated | Ped. 6-6 florally inoculated |
| | | | Acid-hulled seed | Seed in hull | | | | |
| Soil | Air | | Mycelium on agar | Mycelium on wheat | | | | |
| | | Total No. | 278 | 246 | 257 | 230 | 71 [a] | 110 [a] |
| 8 | 12 | Striped No. | 156 | 199 | 174 | 153 | 11 | 14 |
| | | Striped per cent | 56.1 | 80.9 | 67.7 | 66.5 | 15.5 | 12.7 |
| | | Total No. | 291 | 213 | 247 | 196 | 77 [a] | 163 |
| 12 | 12 | Striped No. | 142 | 177 | 113 | 101 | 23 | 21 |
| | | Striped per cent | 48.8 | 83.1 | 45.7 | 51.5 | 29.9 | 12.9 |
| | | Total No. | 320 | 343 | 235 | 248 | 167 | 265 |
| 16 | 16 | Striped No. | 140 | 229 | 47 | 63 | 60 | 56 |
| | | Striped per cent | 43.8 | 66.8 | 20.0 | 25.4 | 35.9 | 21.1 |
| | | Total No. | 287 | 359 | 189 | 209 | 181 | 207 |
| 20 | 16 | Striped No. | 114 | 201 | 17 | 23 | 41 | 31 |
| | | Striped per cent | 39.7 | 56.0 | 9.0 | 11.0 | 22.7 | 15.0 |
| | | Total No. | 200 | 287 | 162 | 166 | 116 | 167 |
| 24 | 24 | Striped No. | 94 | 155 | 7 | 14 | 26 | 25 |
| | | Striped per cent | 47.0 | 54.0 | 4.3 | 8.4 | 22.4 | 15.0 |
| | | Total No. | 120 | 264 | 127 | 141 | 95 | 159 |
| 28 | 28 | Striped No. | 30 | 78 | 0 | 3 | 9 | 4 |
| | | Striped per cent | 25.0 | 29.5 | 0 | 2.1 | 9.5 | 2.5 |

[a] Only one series represented.

The constant-temperature studies were continued in the winter of 1932–1933, and larger populations of plants were used. In addition to the 2 types of inoculations previously employed, kernels were inoculated by growing the fungus on autoclaved wheat kernels for 4 days, followed by placing barley seed in contact with the inoculum and allowing this mixture to incubate for 4 days. Acid-hulled Pedigree 6-6 seed, C.I. 5024, and White Hulless barley were inoculated by placing seed between layers of the agar cultures 21a1, 38, and Ariz. 1, respectively. Pedigree 6-6 Oderbrucker and X76–2–6–1, an Arlington Awnless type, florally inoculated were used (Table 3). There was 83.1 per cent stripe at 12° with a little less at 8°, and still less as the temperature increased, when culture 21a1, grown on wheat, was used as inoculum with Pedigree 6-6 nonhulled as the host. When acid hulled Pedigree 6-6 was inoculated with isolation 21a1, the results coincided with those of the previous winter, namely, a relatively high percentage of stripe at 8°, with less at 12, 16, and 20°, and more again at 24° C. The stripe reaction of C.I. 5024 was somewhat changed; the most disease developed at 8°, 67.7 per cent, and less as the temperature rose. The White Hull-less variety, infected with Ariz. 1 culture of the fungus, gave final infection percentages essentially similar to those of C.I. 5024. The 2 florally inoculated varieties gave still different results in that most striping occurred at the intermediate temperatures, with a decrease in stripe at both the lower and higher temperatures.

The weighted averages of the stripe percentages obtained during the 2 seasons' study in the controlled-temperature tanks are given in table 4. Acid-hulled Pedigree 6-6 seed inoculated with culture 21a1 developed 54.2 per cent stripe at 8°, and 50.7 per cent at 24°, with less at the intermediate temperatures and at 28°. The two hull-less varieties gave most stripe at the lowest temperature used; namely, 8°. When culture 21a1 of the fungus growing on wheat kernels was used as inoculum with Pedigree 6–6 barley kernels with the hulls intact, the highest percentage of disease occurred at 12° with diminishing amounts at the higher temperatures. The two florally inoculated varieties, summarized together, showed most striping near 16°. The results, however, would have been more convincing had greater infection occurred. Thus it follows that the method of inoculation, host variety, and the culture of the parasite have to be considered in stating the temperature which is likely to produce the most stripe. A composite average of the percentages obtained in the two years' study rather clearly showed that most striping was likely to develop at the lower temperatures when barley seed was artificially inoculated with mycelium of *H. gramineum*.

Time is a factor to be taken into account in analyzing the effect of the several temperatures upon the development of stripe. Frequent periodic harvestings of striped plants were made in the first series of 1932–1933 in

TABLE 4.—*Influence of soil and air temperatures on stripe. Summary of data obtained during 1931, 1932, and 1933*

| Method of inoculation | Host variety | Culture of parasite | Percentage of striped plants grown at | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | °C. 8 | °C. 12 | °C. 16 | °C. 20 | °C. 24 | °C. 28 |
| Floral | × 76–2–6–1 and Ped. 6–6 | Conidial suspension | 13.5 | 17.4 | 26.9 | 20.3 | 18.6 | 4.4 |
| Seed incubated with mycelium on wheat | Ped. 6–6 | 21a1 | 80.9 | 83.1 | 66.7 | 56.0 | 54.0 | 29.2 |
| Acid-hulled seed in contact with mycelium on agar | Ped. 6–6 | 21a1 | 52.8 | 46.9 | 44.8 | 45.3 | 50.6 | 32.7 |
| Seed in contact with mycelium on agar | C.I. 5024 | 38 | 61.2 | 54.0 | 25.8 | 17.8 | 12.5 | 3.8 |
| Seed in contact with mycelium on agar | White Hull-less | Ariz.1 | 66.5 | 51.5 | 25.4 | 11.0 | 8.4 | 2.1 |
| Composite average | | | 54.8 | 50.6 | 37.9 | 30.1 | 28.8 | 14.4 |

an attempt to learn more accurately the time after inoculation when certain percentages of stripe appeared. The determination of the time in days required for the appearance of disease symptoms in the first half of the total of striped plants developing at the various soil and air temperatures in the first series was arbitrarily chosen as representing fairly accurately the rate of striping (Table 5). For each method of inoculation the slowest disease production resulted at 8°, with each succeeding higher temperature, including 20°, inducing more rapid striping than the next lower temperature. At 24° the rate of striping was about like that at 20°, except for minor changes. Although the data are not conclusive, the rate of striping seemed to be retarded at 28°. If the percentage of stripe is divided by the number of days required for the first half of the total striped plants to develop symptoms, securing in this way what may be termed a per cent-time index, the data show that 8° gave the highest index for C.I. 5024, 16° for White Hull-less, 24° for acid-hulled Pedigree 6-6, and 20–24° for the floral inoculations. High indices were obtained from 12 to 24° C. for Pedigree 6-6 when inoculated with fungus growing on wheat. The results may be stated in another way; by balancing the percentage of stripe against the rate of development, a "corrected optimum" may be determined, which

lies near 12–16° for the composite grouping of the methods of inoculation, host varieties, and cultures of the parasite.

TABLE 5.—*Time in days required for appearance of disease symptoms in half of the total striped plants developing at the various soil temperatures. Series I, 1932–1933*

| Method of inoculation | Host variety | Culture of parasite | Number of days required for 50 per cent of total stripe to develop at temperatures of | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | °C. 8 | °C. 12 | °C. 16 | °C. 20 | °C. 24 | °C. 28 |
| Floral | × 76–2–6–1 and Ped. 6–6 | Conidial suspension | 65 | 51 | 41 | 35 | 38 | 50 |
| Seed incubated with mycelium on wheat | Ped. 6–6 | 21a1 | 47 | 32 | 28 | 24 | 25 | 27 |
| Acid-hulled seed in contact with mycelium on agar | Ped. 6–6 | 21a1 | 51 | 36 | 28 | 28 | 26 | 27 |
| Seed in contact with mycelium on agar | C.I. 5024 | 38 | 38 | 28 | 21 | 19 | — | — |
| Seed in contact with mycelium on agar | White Hull-less | Ariz.1 | 56 | 37 | 21 | 21 | 19 | — |

*Relation of Change in Temperature.* Because the plants at 8° C. were quite slow in striping in the first constant temperature series of the winter of 1931–1932, one can of plants was placed in a 24° house, where disease soon became evident, with a higher percentage of striped plants than in those held at 8°. This suggested the importance of a study involving periodic change in temperature at various stages of plant and disease development. Leukel *et al.* (8) state . . . "that the influence of soil temperature on the development of stripe does not extend beyond the time of emergence." Three experiments were conducted; 2 in the season of 1931–1932, and a third in the winter of 1932–1933 with the object of determining the effect of temperature change on stripe development when acid-hulled Pedigree 6-6 seed was inoculated with culture 21a1. The kernels were inoculated by placing them between layers of potato-dextrose agar on which the fungus was growing, and were planted in 8-inch pots. Immediately after they were placed in greenhouses with air temperatures of 8–12° and 24°. In the first two series, pots also were placed in a 16° house. Plants started at 8–12° were changed to 24° at periodic intervals, and *vice versa*. Those started at 16°

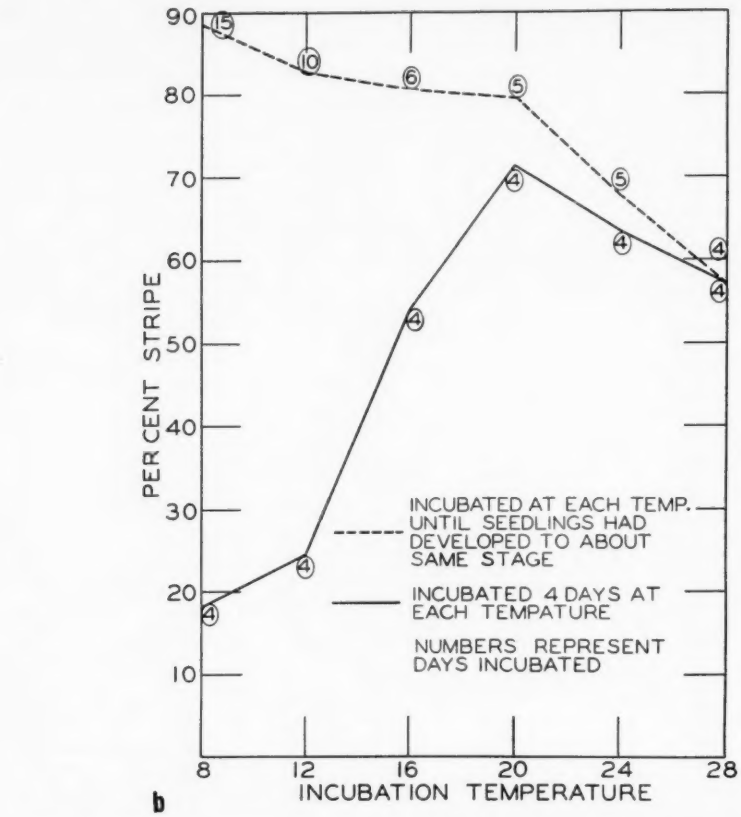
were changed to both 8–12°, and 24°. As a check for each of the series, some of the plants were maintained at constant temperatures. The detailed data of the second series, which was representative of the 3 experiments, are recorded in table 6.

TABLE 6.—*Effect of temperature change upon the development of stripe with acid-hulled Ped. 6–6 seed, inoculated with mycelium of culture 21a1*

| Temp. at which started, or at which grown. °C. | Temp. to which changed. °C. | Days after inoc. when change was made | Total number of plants striped at following days after inoculation | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 43 | 46 | 53 | 59 |
| 10–12 | 24 | 5 | 2 | 7 | 10 | 21 | 30 | 36 | 43 | 47 | 51 | 53 | 54 | 56 | 56 | 56 |
| 10–12 | 24 | 12 | | | 4 | 15 | 19 | 28 | 32 | 35 | 35 | 38 | 38 | 38 | 38 | 40 |
| 10–12 | 24 | 21 | | | | | | 9 | 18 | 25 | 28 | 32 | 33 | 33 | 35 | 35 |
| 10–12 | 24 | 30 | | | | | 1 | 5 | 14 | 33 | 43 | 51 | 52 | 52 | 54 | 57 |
| 10–12 | Not changed | | | | | | 1 | 6 | 11 | 22 | 27 | 31 | 36 | 37 | 42 | 44 |
| 16 | 10–12 | 8 | | | | 1 | 2 | 2 | 5 | 10 | 15 | 17 | 20 | 20 | 22 | 26 |
| 16 | 24 | 8 | | 1 | 7 | 12 | 16 | 20 | 21 | 23 | 23 | 23 | 23 | 24 | 24 | 25 |
| 16 | 10–12 | 18 | | | | | 1 | 6 | 11 | 16 | 20 | 22 | 22 | 22 | 22 | 25 |
| 16 | 24 | 18 | | | 2 | 5 | 10 | 32 | 37 | 39 | 42 | 43 | 44 | 46 | 46 | 47 |
| 16 | Not changed | | | | | 1 | 6 | 21 | 24 | 27 | 27 | 27 | 27 | 28 | 28 | 30 |
| 24 | 10–12 | 3 | | | | | 1 | 3 | 16 | 24 | 30 | 33 | 38 | 41 | 47 | 47 |
| 24 | 10–12 | 7 | | | | | | 2 | 3 | 12 | 15 | 18 | 20 | 21 | 25 | 26 |
| 24 | 10–12 | 13 | | 2 | 2 | 2 | 4 | 6 | 12 | 15 | 22 | 25 | 27 | 27 | 28 | 29 |
| 24 | 10–12 | 18 | 1 | 4 | 11 | 15 | 19 | 22 | 32 | 38 | 42 | 44 | 46 | 46 | 47 | 48 |
| 24 | Not changed | | 1 | 4 | 8 | 14 | 22 | 24 | 28 | 31 | 32 | 34 | 35 | 35 | 37 | 37 |

As suggested in earlier experiments, stripe developed slowly at 8°, and very much faster at 24°, when the temperatures remained constant. When the inoculated plants were changed from lower to higher temperatures, the rate of disease appearance was stimulated, and when shifted from higher to lower temperatures, striping was retarded. When inoculated plants started at an intermediate temperature were changed to the lower one, development of symptoms was slower, and when changed to 24°, striping was much faster.

The time of disease appearance and the percentage of total stripe of the 3 series are summarized in table 7, and illustrated graphically in figure 2a. Group No. 1 was earliest in striping, developing 25, 50, and 75 per cent stripe in 18, 24, and 30 days, respectively. This group represents those plants started at a low temperature and shifted to the higher one 5–9 days after inoculation. Group No. 2, shifted from low to high temperature 12–14


PER CENT STRIPE
INCUBATED AT EACH TEMP. UNTIL SEEDLINGS HAD DEVELOPED TO ABOUT SAME STAGE
INCUBATED 4 DAYS AT EACH TEMPATURE
NUMBERS REPRESENT DAYS INCUBATED
INCUBATION TEMPERATURE
b

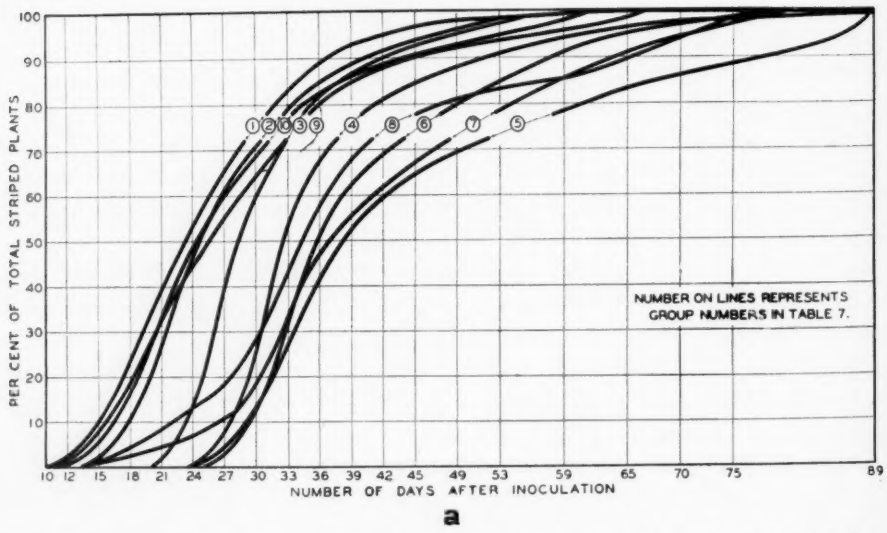



FIG. 2. The effect of temperature change and incubation period on stripe. (a) Influence of temperature change upon stripe development using acid-hulled Ped. 6-6 seed, inoculated with mycelium of culture 21a1. Summary of three series. (b) Effect of incubation temperature on stripe in Ped. 6-6 barley when inoculated with culture 21a1 grown on wheat.

TABLE 7.—*Effect of temperature change on stripe development using acid-hulled Ped. 6-6 seed, inoculated with culture 21a1. Summary of 3 series*

| Group No. | Temp. at which started or at which grown °C. | Temp. to which changed °C. | Days after inoc. when change was made | Percentage of striped plants at following days after inoculation | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 49 | 53 | 59 | 65 | 70 | 75 | 89 |
| 1 | 8–12 | 24 | 5–9 | 2 | 15 | 23 | 34 | 53 | 66 | 76 | 83 | 87 | 91 | 93 | 98 | 98 | 98 | 98 | 100 | 100 | 100 | 100 |
| 2 | 8–12 | 24 | 12–14 | | | 10 | 29 | 48 | 63 | 73 | 78 | 81 | 87 | 87 | 94 | 98 | 98 | 99 | 99 | 99 | 100 | 100 |
| 3 | 8–12 | 24 | 16–22 | | | | 1 | 16 | 39 | 56 | 76 | 81 | 87 | 89 | 92 | 97 | 100 | 100 | 100 | 100 | 100 | 100 |
| 4 | 8–12 | 24 | 30–42 | | | | | 1 | 6 | 27 | 51 | 64 | 74 | 83 | 85 | 88 | 89 | 98 | 98 | 98 | 98 | 100 |
| 5 | 8–12 | Not changed | | | | | | 1 | 6 | 19 | 30 | 39 | 49 | 53 | 62 | 70 | 70 | 78 | 81 | 83 | 85 | 100 |
| 6 | 24 | 8–12 | 2–5 | | | | | 1 | 4 | 23 | 35 | 45 | 53 | 59 | 70 | 81 | 81 | 92 | 96 | 96 | 97 | 100 |
| 7 | 24 | 8–12 | 7–9 | | 1 | 2 | 3 | 6 | 10 | 17 | 30 | 38 | 50 | 52 | 68 | 75 | 75 | 86 | 91 | 95 | 98 | 100 |
| 8 | 24 | 8–12 | 12–16 | | 2 | 3 | 8 | 11 | 17 | 25 | 43 | 55 | 68 | 74 | 75 | 80 | 80 | 86 | 91 | 95 | 99 | 100 |
| 9 | 24 | 8–12 | 18–22 | 2 | 8 | 23 | 32 | 41 | 45 | 62 | 71 | 82 | 85 | 88 | 89 | 89 | 91 | 94 | 97 | 100 | 100 | 100 |
| 10 | 24 | Not changed | | 1 | 5 | 16 | 34 | 51 | 59 | 70 | 81 | 82 | 85 | 86 | 91 | 92 | 94 | 98 | 100 | 100 | 100 | 100 |

days after inoculation, practically paralleled the stripe development in group No. 10, held constant at 24°; both were slightly later than No. 1. Group No. 9, changed from 24° to the lower temperature 18–22 days after inoculation was still a little later in striping than any of the groups previously discussed. Groups 3 and 4, shifted from low to high 16–22, and 30–42 days after inoculation were still slower in the initiation of striping; but thereafter, were much faster than any of the other groups, whether changed or constant. Groups 8 and 7, moved from high to low temperature at 12–16, and 7–9 days after inoculation, though striping began relatively soon, were quite slow in the relative rate of disease appearance, requiring 30 and 32, 38 and 39, and 49 and 50 days, respectively, for 25, 50, and 75 per cent stripe to occur. Group 6, shifted from a high to a low temperature 2–5 days after inoculation, reacted almost like that of group 5, which was held constantly at 8–12°, and was slowest in rate of striping. These results have been substantiated by using Pedigree 6–6 seed inoculated by placing it in contact with mycelium of culture 21a1 growing on sterilized wheat, followed by changes at periodic intervals from low to high, and from high to low temperatures.

The data accumulated with reference to the influence of temperature change upon the final percentage of striped plants appearing, presented in table 8, rather definitely indicated that less stripe developed when inoculated plants were changed from high to low temperatures than when shifted from low to high temperatures. In each of the first 4 groups changed from low to high, where the three experiments are considered collectively, the final percentage of stripe was greater than in any of groups 6–9 inclusive, which were started at 24° and later changed to 8–12°. This is likewise true of the 3 series taken separately, with slight exceptions in the third series. In comparing the amount of striping in each series, the highest disease percentage was in the first, with less in the 2 later series.

### INFLUENCE OF INCUBATION TEMPERATURE ON STRIPE DEVELOPMENT

The investigation of the influence of incubation temperature on stripe development was approached from 2 angles: (1) seedlings were incubated after inoculation 4 days at the respective temperatures, after which the plants were grown in a greenhouse held at 16° C.; and (2) seedlings were inoculated and incubated until they reached approximately the same stage of development at each temperature, after which the plants were likewise grown in a greenhouse held at 16°. Pedigree 6–6 barley, inoculated with culture 21a1 growing on sterilized wheat kernels was used in the 2 duplicated experiments (Fig. 2b). The results of the 2 series incubated during a 4-day period at the several temperatures are given in table 9. The most stripe, 71.5 per cent, developed at 20 °, less as the temperature increased or

TABLE 8.—*The influence of temperature change on the final percentages of stripe development*

| Group No. | Temp. at which started or at which grown °C. | Temp. to which changed °C. | Days after inoc. when change was made | Series I | | | Series II | | | Series III | | | Total plants used | | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Plants used | | | Plants used | | | Plants used | | | | | |
| | | | | Total No. | Striped No. | Striped per cent | Total No. | Striped No. | Striped per cent | Total No. | Striped No. | Striped per cent | Total No. | Striped No. | Striped per cent |
| 1 | 8–12 | 24 | 5–9 | 42 | 38 | 90.5 | 102 | 56 | 54.9 | | | | 144 | 94 | 65.3 |
| 2 | 8–12 | 24 | 12–14 | 29 | 22 | 75.9 | 91 | 40 | 44.0 | 90 | 41 | 45.6 | 210 | 103 | 49.0 |
| 3 | 8–12 | 24 | 16–22 | 62 | 44 | 71.0 | 77 | 35 | 45.5 | | | | 139 | 79 | 56.8 |
| 4 | 8–12 | 24 | 30–42 | 63 | 43 | 68.2 | 105 | 57 | 54.3 | 90 | 40 | 44.4 | 258 | 140 | 54.3 |
| 5 | 8–12 | Constant | | 88 | 53 | 60.3 | 97 | 44 | 45.4 | 84 | 38 | 45.2 | 269 | 135 | 50.2 |
| 6 | 24 | 8–12 | 2–5 | 58 | 27 | 46.6 | 108 | 47 | 43.5 | | | | 166 | 74 | 44.6 |
| 7 | 24 | 8–12 | 7–9 | 57 | 31 | 54.4 | 86 | 26 | 30.2 | 88 | 39 | 44.3 | 231 | 96 | 41.6 |
| 8 | 24 | 8–12 | 12–16 | 58 | 34 | 58.6 | 91 | 29 | 31.9 | 81 | 38 | 46.9 | 230 | 101 | 43.9 |
| 9 | 24 | 8–12 | 18–22 | 29 | 18 | 62.1 | 114 | 48 | 42.1 | | | | 143 | 66 | 46.2 |
| 10 | 24 | Constant | | 46 | 33 | 71.7 | 95 | 37 | 38.9 | 66 | 48 | 72.7 | 207 | 118 | 57.0 |

decreased, with the least stripe, 18.7 per cent, at 8°. However, when the seedlings were incubated until they reached approximately the same stage of development, the stripe reaction was markedly different (Table 10). The number of days incubated at 28, 24, 20, 16, 12, and 8° was 4, 5, 5, 6, 10, and 15, respectively. The highest disease percentage developed at 8°, with a decrease at each succeeding increase in temperature. By compensating for increased rate of development at the higher temperatures with a longer in-

TABLE 9.—*Influence of incubation temperature on stripe development. Seedlings incubated 4 days at 6 different temperatures; the plants were then grown in a greenhouse at 16° C.*

| Series | Determinations | Temperature in °C. at which incubated | | | | | |
|---|---|---|---|---|---|---|---|
| | | 8° | 12° | 16° | 20° | 24° | 28° |
| I | Total No. | 87 | 91 | 181 | 96 | 80 | 147 |
| | Striped No. | 9 | 21 | 93 | 64 | 56 | 89 |
| | Striped per cent | 10.3 | 23.1 | 51.4 | 66.7 | 70.0 | 60.5 |
| II | Total No. | 95 | 99 | 84 | 90 | 86 | 176 |
| | Striped No. | 24 | 26 | 51 | 69 | 50 | 95 |
| | Striped per cent | 25.3 | 26.3 | 60.7 | 76.7 | 58.1 | 54.0 |
| Average | Total No. | 182 | 190 | 265 | 186 | 166 | 323 |
| | Striped No. | 33 | 47 | 144 | 133 | 106 | 184 |
| | Striped per cent | 18.1 | 24.7 | 54.3 | 71.5 | 63.9 | 57.0 |

TABLE 10.—*Influence of incubation temperature and time on stripe development. Seedlings incubated to approximately same stage of development at each temperature and then maintained in a greenhouse at 16° C.*

| Series | Determinations | Temperature in °C. and number of days incubated before planting | | | | | |
|---|---|---|---|---|---|---|---|
| | | 8° 15 | 12° 10 | 16° 6 | 20° 5 | 24° 5 | 28° 4 days |
| I | Total No. | 79 | 66 | 74 | 86 | 65 | 147 |
| | Striped No. | 72 | 56 | 62 | 72 | 53 | 89 |
| | Striped per cent | 91.1 | 84.8 | 83.8 | 83.7 | 81.5 | 60.5 |
| II | Total No. | 91 | 191 | 99 | 153 | 87 | 176 |
| | Striped No. | 79 | 157 | 78 | 119 | 50 | 95 |
| | Striped per cent | 86.8 | 82.2 | 78.8 | 77.8 | 57.5 | 54.0 |
| Average | Total No. | 170 | 257 | 173 | 239 | 152 | 323 |
| | Striped No. | 151 | 213 | 140 | 191 | 103 | 184 |
| | Striped per cent | 88.8 | 82.9 | 80.9 | 79.9 | 67.8 | 57.0 |

cubation period at the lower temperatures, the resulting stripe was decidedly different from that produced when equal times of incubation periods were used.

## DISCUSSION

In analyzing the effect of temperature on the development of stripe, such reactions as the effect of temperature on the host, on the parasite, and on the combination of the two in the production of disease must be studied. The temperature influence upon either plant alone presents a difficult problem for analysis that is made more complex when considered in relation to the phenomena of disease production. The final response of the host and parasite, or disease development, is the result of a series of interactions that are influenced by the successive stages of infection and parasitic invasion and the various responses on the part of the host. In studying temperature effects in relation to disease, the particular strain of the parasite, the pecularities of the host variety used, the length of incubation period and temperature of incubation are some of the factors that the present study indicates should be taken into account. When constant temperatures alone were considered, the highest final percentage of stripe occurred at the lower temperatures; but when time was taken into account, the highest percentage of stripe per unit of time was found at the intermediate temperatures. When inoculated kernels were incubated until reaching approximately the same stage of development, the stripe reaction was similar to that of the plants held at constant temperatures. However, when the time of incubation was uniform, and temperature alone varied, the stripe reaction was decidedly different from the cases just mentioned in that the greatest percentage of stripe shifted to 20°. The change of temperature markedly affected both time of disease appearance and the rapidity of striping or the development of later symptoms. Whether under greenhouse or field conditions, the net result of infection in relation to temperature of incubation and changes in temperature is not alone measured by the percentage of stripe developing, but also involves the rate of development and severity of disease manifestation. From the practical standpoint, the final result is essentially the same in that all striped plants are worthless for grain production, but from the point of view of studying the influence of temperature upon the successive stages of striping, it is important to attempt a separation of these different factors and integrate them in terms of the detailed steps in disease development.

## SUMMARY

The optimum temperature for the mycelial growth of *Helminthosporium gramineum* on potato-dextrose agar was found to be near 25° with a maximum above 32°, and a minimum considerably below 8° C.

Barley is a relatively low temperature plant, and with constant soil temperatures developed best near 12–16°. At these temperatures, barley developed more slowly than at the higher temperatures such as 20–24°, but the latter temperatures did not promote a balanced growth of the plants.

Floral inoculations were made by spraying a conidial suspension on flowers that had been enclosed within glassine bags about the time the heads emerged from the uppermost leaf sheath. Seed inoculations were made by, (1) placing seed between layers of potato-dextrose agar on which the fungus was growing, and (2), by incubating seed that had been placed in contact with mycelium of the fungus growing on steam-sterilized wheat kernels.

Several types of stripe reaction were evident when the different methods of inoculation, different cultures of the fungus, and different host varieties were used at the constant temperatures. Generally speaking, the lower temperatures induced the greatest percentage of striping when time was left out of consideration. The lower temperatures required longer for striping, which did not permit the definition of a clear-cut optimum. The relationship between time and the percentage of stripe development should be taken into account before definitely assigning an optimum temperature.

Change in temperature from low to high and high to low in the early stages of growth of barley and infection by the parasite markedly affected host and stripe development when seed was inoculated with mycelium. The change from low to high stimulated disease appearance, while the opposite change retarded disease expression. The final percentage of stripe was not greatly altered, but there was a tendency toward higher percentages of stripe when the plants were changed from low to high temperatures than when the opposite change was made.

In studying the influence of incubation temperature upon stripe development, the highest percentage of disease occurred at 20° C. when inoculated seedlings were incubated 4 days at all temperatures and later grown in a greenhouse at 16° C. The greatest percentage of stripe developed at the lower temperatures when inoculated seedlings were incubated until reaching approximately the same stage of development at each temperature and later grown in a greenhouse held at 16° C.

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# SOME FACTORS INFLUENCING GERMINATION OF SPORES OF PHYLLOSTICTA SOLITARIA

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## REVIEW OF LITERATURE

Although the apple-blotch fungus, *Phyllosticta solitaria* E. & E. has been the subject of much investigation, no thorough study of the effect of temperature and other factors on the germination of spores of this organism has yet been made.

Gardner (3) reported that spores of *Phyllosticta solitaria* germinated in water most rapidly at 75° to 80° F. (24° to 27° C.), but readily also at 68° and 61° F. (20° and 16° C.). Guba (4), in connection with considerable experimental work on the apple-blotch fungus, made some studies of spore germination. He used spores from pycnidia on leaves, fruit, and bark, putting them to germinate in sterilized water, tap water, rain water, and water containing crushed pieces of apple peelings, leaves, and bark. He found the percentages of germination to be small, irregular, and generally insignificant. He stated that sterilized distilled water was not a favorable medium, since germination was scant and germ tubes grew slowly, ultimately collapsing from want of nutrient, while in tap water, germination might reach 100 per cent. When spores from culture were placed in apple-fruit juice, weak sugar solution, sterilized water, or apple-bark extract, Guba found best germination in the solution first named and the degree of germination ranged in the order given. Spores, suspended in weak apple extract, from a 32-day old culture, germinated best at 25° C., while in those from a 46-day culture germ-tube development was most extensive and rapid at 25° and 30° C. Guba's results indicate the optimum temperature for spore germination to lie around 30° C. and rate of germ-tube growth to be greatest at 25° and 30° C. At lower temperatures, 15° and 20° C., the amount of germination was at first small and growth of germ tubes slow. Germination rarely occurred at 5° and 10° C. Kohl (5) carried out investigations of spore germination of *P. solitaria*. Sowings were made in various dilutions of citric acid, HCl, $HNO_3$, sodium oxalate, $H_2O_2$, and boiled and fresh decoctions of apple fruits, apple leaves, apple bark, prune decoctions, and sugar solutions. Controls were carried out in sterilized distilled water. There was no evidence that any of the foregoing solutions stimulated germination.

[1] The writer wishes to express her sincere thanks to Dr. A. J. Mix for encouragement and advice during the course of this investigation.

## METHODS

Most of the spores used in this study were obtained from culture; some, however, were taken from twig cankers for purposes of comparison. Viable spores were obtained in culture by growing *Phyllosticta solitaria* on S/10 potato-dextrose agar[2]; and K.U. agar,[3] as recommended by Mix (8), and on K.U. agar with albumen substituted for potassium nitrate.

Cultures were grown in 125 cc. Erlenmeyer flasks, 40 cc. of the medium being placed in each flask. In order to keep a constant supply new cultures were made about every 2 weeks. Since *Phyllosticta solitaria* is a slow-growing fungus it took from 2 to 4 weeks for pycnidia with viable spores to develop. The cultures usually were above 4 weeks old (3 weeks in the case of culture No. 24).

By means of a sterilized needle pycnidia were transferred to a drop of distilled water. These were crushed, allowing the spores to escape into the drop, care being taken to have the spores evenly distributed throughout the liquid, and to have approximately the same number of spores on each slide. In the study of temperature relations spores were at first put to germinate in small drops of liquid on slides, but this method proved unsatisfactory, since the liquid evaporated at high temperatures. Consequently, the paraffin-ring method, allowing the use of larger drops of liquid, was devised. The slides were cleaned carefully but not sterilized and no special care was taken to use perfectly sterile liquid. They were then placed in Petri dishes that had been lined with moist filter paper.

For spores secured from nature, essentially the same methods were used. Pycnidia with a little adherent bark were removed from twigs by means of a scalpel and placed on a slide. The bark was cut or pycnidia crushed so as to free the spores into the liquid and the latter were placed in liquid on paraffin-ringed slides.

For the study of the effect of various nutrients on spore germination such solutions as rain water, tap water, distilled water, potato-dextrose broth, apple-fruit juice, bark decoction, K.U. solution, and modified Coons' solution were tried. The apple juice was made by boiling 70 grams of ripe apple in 500 cc. distilled water, filtering, and sterilizing. Bark decoction (50 grams of bark of apple twigs to 1000 cc. distilled water heated, then allowed to stand over night, and filtered) also was sterilized. The K.U. solution was made in the same way as the K.U. agar mentioned above, omitting the agar. The modified Coons' solution consisted of $KNO_3$ 2.02 gm., $KH_2PO_4$ 2.72 gm., $MgSO_4$ 1.25 gm., dextrose 10 gm., distilled water

[2] Standard potato-dextrose broth: potatoes 140 gm., dextrose 10 gm., water 1000 cc., the whole being diluted to 1/10 its original strength and 20 grams of agar added.

[3] K.U. agar: $KNO_3$ 0.5 gm., $KH_2PO_4$ 1.25 gm., $MgSO_4$ 0.75 gm., dextrose 1 gm., agar 20 gm., $H_2O$ 1000 cc.

1000 cc. In these studies the spores were suspended in hanging drops of the solution in microslide cells. The slides were placed in Petri dishes with moist filter paper and kept at room temperature. All cover glasses, microcells, Petri dishes, and solutions were sterilized and every precaution was taken to prevent contamination.

### EFFECT OF LIGHT AND DARK

Spores were sown in distilled water on slides in moist Petri dishes. One dish was then left in the light at room temperature and the other, wrapped with black paper, was placed in a drawer at room temperature. In agreement with Guba's results the writer found no significant difference between light and dark. Seventy-five per cent of the spores placed in the light germinated, while but 68 per cent of those in the dark germinated. Similar results obtained when the experiment was repeated.

### EFFECT OF TEMPERATURE

For this study the Petri dishes containing the slides were placed in dark chambers (light and dark, as shown above, having no apparent effect on germination) in a large temperature tank modeled after that described by Livingston and Fawcett (7). After preliminary adjustment each chamber in this tank assumes a different temperature and the temperatures remain constant until the range of temperatures is changed.

For the temperature studies spores were put to germinate in distilled water. Preliminary trials of this and other liquids resulted in somewhat better germination in potato-dextrose broth. This medium, however, becomes readily contaminated, and the use of distilled water obviated the necessity of sterilization. Distilled water allows from 90 to 98 per cent germination.

The spores of the apple-blotch fungus germinate rather slowly. Lewis (6) has reported that they germinate in the laboratory in from 12–18 hours and Scott and Rorer (10) report that they germinate in 15 hours. In this present study spores were examined on the second day, since it was found that earlier examination gave much lower counts. In determining the percentages of germination shown in table 1, experiment 1, from 100 to 200 spores, usually 150, were counted in each case. For table 1, experiment 2, the counts were of from 50 to 100 spores, ordinarily 100, while for table 2 approximately 50 spores were counted each time. Besides percentage of germination the average length of germ tube was calculated in each case. Each germ tube was measured carefully by means of an eyepiece micrometer and the sum of germ-tube lengths of all germinated spores was divided by the number of spores counted. Average germ-tube length, calculated

in this way, is recommended by Brown (2) as a better measure of the rate of early growth of a fungus than percentage of germination.

In table 1, experiment 1, the results of 3 trials are recorded. In the first trial the spores were counted the second day, while in the other 2 trials a count was made the second and fifth days. At a temperature of 5°–6° C. and at 34°–36° C. there was, in most cases, a very low percentage of germination. Even at 12°–14° C. and 29°–31° C. there was a comparatively low germination. The optimum temperature was 21°–23° C. but germination at 25°–27° C. was nearly as good. In the second experiment, reported in table 1, a slightly different range of temperatures was employed. This table shows a low percentage of germination at 8°–10° C. and a still lower one at 37°–38° C. The optimum in this case occurs at about 21°–22° C., although the percentages also are quite high at 25°–27° C.

The data secured by measurement of germ-tube lengths are presented in table 1, on the right. The germ-tube lengths were measured in microns. There was no appreciable difference in width of germ tubes. The average germ-tube length was shorter at the extreme low and high temperatures where there was a lower percentage of germination. In table 1, experiment 1, 3 of the 5 counts made show the greatest germ-tube length at 21°–23° C. the other 2 showing better at 25°–27° C. Also, in table 1, experiment 2, the optimum growth is found at 21°–22° C. and in table 2 the best germ-tube-length average is at 23° C. It seems that, in general, the highest percentage of germination and greatest average germ-tube growth agree.

The lowest and highest temperatures used in this study, namely 5° and 39° C., are believed to be close to the minimum and maximum temteratures for germination. It will be seen, however, that no temperature used completely inhibited germination. The optimum temperature (of those employed) is 21°–23° C. The actual optimum may well be at 24° C., since this temperature was not tried. The discrepancy between the author's results and those of Guba (4) cannot well be explained. It may be stated, however, that Guba's experimental data are very meager. The results here reported more nearly agree with those of Gardner (3), who found the optimum for germination to lie between 24° and 27° C.

Apple blotch is a disease of the central United States, being less prevalent in the northern part of its range and absent from extreme northern localities. It was thought an explanation of this fact might be found in the temperature requirements for spore germination. From these results, however, it is clear that no such limiting temperature requirements exist. Mix (8) has shown that the temperature requirements for spore production in culture do not afford an explanation of the present distribution of the fungus. Perhaps a study of the effect of temperature on infection by

TABLE 1.—*Effect of temperature on germination of spores of Phyllosticta solitaria from culture*

FIRST EXPERIMENT

| Temperature | Trial number | Germination | | Average length of germ tubes | |
|---|---|---|---|---|---|
| ° *C.* | | *Per cent* | | *Microns* | |
| | After | 48 hrs. | 120 hrs. | 48 hrs. | 120 hrs. |
| | 1 | 15 | ..... | 2.32 | ..... |
| 5– 6 ..... | 2 | 2 | 6 | 0.14 | 0.61 |
| | 3 | 1 | 3 | 0.21 | 0.29 |
| | 1 | 37 | ..... | 8.37 | ..... |
| 12–14 ..... | 2 | 10 | 43 | 1.27 | 9.27 |
| | 3 | 9 | 22 | 1.01 | 2.66 |
| | 1 | 37 | ..... | 6.77 | ..... |
| 17–19 ..... | 2 | 32 | 55 | 5.78 | 9.17 |
| | 3 | 17 | 52 | 2.42 | 5.87 |
| | 1 | 84 | ..... | 22.18 | ..... |
| 21–23 ..... | 2 | 49 | 70 | 9.15 | 9.36 |
| | 3 | 44 | 62 | 6.49 | 5.52 |
| | 1 | 77 | ..... | 19.82 | ..... |
| 25–27 ..... | 2 | 47 | 65 | 9.81 | 7.98 |
| | 3 | 37 | 55 | 4.46 | 6.24 |
| | 1 | 46 | ..... | 9.92 | ..... |
| 29–31 ..... | 2 | 13 | 25 | 2.09 | 3.30 |
| | 3 | 15 | 29 | 2.11 | 3.36 |
| | 1 | 23 | ..... | 3.96 | ..... |
| 34–36 ..... | 2 | 3 | 3 | 0.31 | 0.25 |
| | 3 | 6 | 9 | 0.77 | 1.10 |

SECOND EXPERIMENT

| Temperature | Trial number | Germination | | | Average length of germ tubes | | |
|---|---|---|---|---|---|---|---|
| ° *C.* | | *Per cent* | | | *Microns* | | |
| | After | 48 hrs. | 96 hrs. | 120 hrs. | 48 hrs. | 96 hrs. | 120 hrs. |
| 8–10 ..... | 1 | 10 | 18 | ..... | 0.72 | 3.05 | ..... |
| | 2 | 35 | ..... | 40 | 6.63 | ..... | 9.58 |
| 15–17 ..... | 1 | 48 | 67 | ..... | 9.69 | 14.10 | ..... |
| | 2 | 60 | ..... | 72 | 12.59 | ..... | 13.65 |
| 21–22 ..... | 1 | 82 | 86 | ..... | 18.74 | 16.99 | ..... |
| 25–27 ..... | 1 | 62 | 79 | ..... | 10.92 | 14.40 | ..... |
| 29–31 ..... | 1 | ..... | 55 | ..... | ..... | 7.50 | ..... |
| 37–39 ..... | 1 | 3 | 5 | ..... | 0.22 | 0.57 | ..... |
| | 2 | 15 | ..... | 17 | 1.61 | ..... | 1.01 |

*Phyllosticta solitaria* will furnish the desired information. Pending such a study it is necessary to fall back on the explanations offered by Gardner (3) : a shorter growing season in the north unfavorable to mycelial growth, insufficient time for invasion of twigs by the fungus from petiole lesions, or retarded growth of cankers and production of spores in the spring.

Until these further possibilities are eliminated it is well to keep in mind the opinion of Anderson (1) that apple blotch may ultimately become serious in northern localities.

### COMPARISON OF SPORES FROM NATURE AND FROM CULTURE

Although Kohl (5) reports only one experiment, he seems to have experienced much difficulty in obtaining germination of spores formed in culture. He states "it is conceivable that the common lack of viability of spores in culture may be attributable in part to injury by drying, since it requires approximately 35 days for the spores to mature in tube cultures." Apparently, Kohl means that this injury results from the desiccation of pycnidia and spores in old cultures, but it should be noted that elsewhere in the same paper he reports the occurrence of spores in 20- to 22-day-old cultures.

Since both Mix (8) and the writer have found abundant sporulation in cultures that had not begun to dry, there seems to be no point to Kohl's suggestion. However, in view of his experience, it seemed desirable to compare the germination of spores from culture with those from nature. Spores from both sources, therefore, were put to germinate at the same time in the same temperature chambers. The same general trend of germination (Table 2) was found in this experiment as in previous ones: that is, there was a lower percentage of germination at 15°–16° C. of spores from nature, as well as those from culture, and the optimum was 21°–23° C. in both cases. Spores from nature, however, showed consistently lower germination than those from culture. The former were obtained from cankered twigs about the first of June, 1932. Two other comparisons of spores from nature and from culture were made. On May 9 spores obtained from cankers were put to germinate at room temperature along with spores from culture. The former gave 54–60 per cent germination as compared with

TABLE 2.—*Effect of temperature on germination (in 48 hours) of spores of Phyllosticta solitaria from culture and nature*

| Temperature | From culture | | From nature | |
|---|---|---|---|---|
| | Germination | Average length of germ tubes | Germination | Average length of germ tubes |
| ° *C.* | *Per cent* | *Microns* | *Per cent* | *Microns* |
| 15–16 | 17 | 1.90 | 4 | 0.65 |
| 17 | 59 | 13.04 | ..... | ..... |
| 18–19 | 68 | 17.90 | 11 | 0.79 |
| 23 | 84 | 20.03 | 15 | 1.73 |
| 25 | 60 | 10.99 | 12 | 0.70 |

80–90 per cent germination of the latter. Again, on June 2, 36.8 per cent of spores taken from pycnidia on cankered twigs germinated at room temperature, while those from culture gave 56.6 per cent germination.

It may be concluded that spores from culture germinate quite as well as those from nature. The consistently higher germination of spores from culture obtained in these experiments may perhaps be explained on the basis that these spores were physiologically mature, while those obtained from twig cankers were not. (A discussion of physiological maturity of spores will be found later in this paper.)

### EFFECT OF VARIOUS NUTRIENTS

Table 3 shows the effects of various nutrients on the germination of spores of *Phyllosticta solitaria*. Of the solutions used bark decoction allowed the best germination. Potato-dextrose broth also gave fairly high germination. K. U. solution and tap water, compared with distilled water, showed an inhibitory effect. (Tap water in this laboratory frequently is chlorinated.) Coons' solution caused a slight increase over distilled water. In table 3, on the right, are recorded the germ-tube lengths for spores placed in the various solutions. Usually the best solution for germination also was best for germ-tube growth. Bark-decoction and potato-dextrose broth enhanced the latter.

### EFFECT OF MATURITY OF SPORES

Guba (4) believes that, in addition to moisture, nutrition, and proper temperature, maturity of spores is necessary for germination, stating that spores from young spore-bearing cultures fail to germinate. He found that less than 1 per cent of the spores from 16-day-old cultures germinated; and, by further comparison of 32-day-old and 46-day-old cultures, he decided that as the pycnidia aged the percentage of germination increased. The highest germination occurred when spores were taken from 1- to 1½-month-old cultures. From April 15 to July 15, Guba made repeated attempts to germinate spores from pycnidia on leaves, fruit, and bark, but with very little success. Roberts (9) tested germination of spores from bark cankers at various dates, but the number of spores germinating was too small to allow conclusions as to date of maturity. He obtained germination in distilled water after May 23, 1914; on May 13, 1915, he found 10 per cent, and after May 24, 75 per cent had germinated.

Kohl (5) offers the same reason for the lack of viability of spores. He believes that in nature and in culture there is an interval between morphological and physiological maturity, and states, without reporting any experiments, that this interval could not be shortened in culture by artificial means. He reports that, in southern Indiana, spores in twig cankers are

TABLE 3.—*Effect of various nutrients on germination of spores of Phyllosticta solitaria from culture*

| Medium | Trial number | Germination | | Average length of germ tubes | |
|---|---|---|---|---|---|
| | | *Per cent* | | *Microns* | |
| | After | 48 hrs. | 96 hrs. | 48 hrs. | 96 hrs. |
| Distilled water | 1 | ..... | 8 | ..... | 0.70 |
| | 2 | 35 | 62 | 5.88 | 10.27 |
| | 3 | 23 | 55 | 4.23 | 10.96 |
| Potato-dextrose broth | 1 | 32 | 89 | 3.92 | 58.61 |
| | 2 | 79 | 80 | 14.70 | 28.84 |
| | 3 | 53 | 68 | 5.56 | 17.09 |
| Coons' solution | 1 | 0 | 37 | 0.00 | 2.54 |
| | 2 | 21 | 29 | 1.03 | 2.24 |
| | 3 | 27 | 57 | 2.03 | 11.86 |
| Rain water | 1 | 0 | 2 | 0.00 | 0.03 |
| | 2 | 11 | 24 | 1.38 | 1.88 |
| | 3 | 11 | 30 | 1.93 | 4.13 |
| Tap water | 1 | 1 | 5 | 0.03 | 0.62 |
| | 2 | 10 | 12 | 0.71 | 1.29 |
| | 3 | 4 | 22 | 0.62 | 7.80 |
| K. U. solution | 1 | 0 | 1 | 0.00 | 0.03 |
| | 2 | 10 | 17 | 1.50 | 12.00 |
| | 3 | 4 | 6 | 0.34 | 0.30 |
| Apple-fruit juice | 1 | ..... | ..... | ..... | ..... |
| | 2 | 11 | 29 | 1.17 | 3.84 |
| | 3 | 2 | 18 | 0.06 | 2.04 |
| Apple-bark decoction | 1 | ..... | ..... | ..... | ..... |
| | 2 | 73 | 96 | 16.68 | 98.93 |
| | 3 | 63 | 92 | 6.25 | 31.44 |
| Distilled water + apple-fruit tissue | 1 | ..... | ..... | ..... | ..... |
| | 2 | ..... | 2 | ..... | 0.19 |
| Distilled water + apple bark | 1 | ..... | ..... | ..... | ..... |
| | 2 | ..... | 86 | ..... | 53.35 |
| | 3 | ..... | 80 | ..... | 38.37 |

ordinarily morphologically mature about April 1 and physiologically mature by May 1.

In the present investigation study was made of the time of maturation of spores in culture. Pycnidia, as soon as they appeared, which was usually when the cultures were about 4 weeks old, were examined for spores. When first examined they contained droplets of protoplasm and no spores. In nearly every case it took from 4 to 7 days longer for spores to appear. Frequently, from one flask to another, there occurred a slight variation in the maturity of spores and their capacity to germinate. For example, in one flask 30 per cent of the spores germinated, while spores taken from a dupli-

cate flask showed only 20 per cent germination (Table 4). After the spores had matured they were put to germinate in distilled water at room temperature. They were examined on the 2nd and 4th days. This process, involving several cultures, was repeated at frequent intervals to allow, where necessary, for further maturity of spores (Table 4). In nearly every case, spores only one day old gave a low percentage of germination. With the advancing age of the cultures there was an increase in the percentage germination, the time of maximum germination varying somewhat with different cultures. In culture 299 the highest percentage of germination was found when the spores were 19 and 22 days old. In culture 342 the maximum was on the 15th day and in the other cultures it occurred on the 11th day. It seems that a period of maturation is necessary for best spore germination, although some germination occurs as soon as the spores are formed. Thirty-day-old spores show low percentages of germination and a little later they may not be found in cultures. Mix (8) also records this autolysis of spores occurring in old cultures. Table 4 gives the germ-tube lengths and the results show that the optimum time for germination usually is the best for germ-tube growth.

It may be concluded that a period of maturation is necessary to the best germination of spores from culture, although their period is shorter than Kohl seems to have observed. The period between the first appearance of spores and their maximum germination is relatively short. Since spores develop in culture in about 4 weeks and the most profuse germination is approximately 2 weeks later, spores taken from 1½-month-old cultures give best results. This in in agreement with Guba's report.

A few observations were made as to time of maturity of spores in nature. Pycnidia from twig-cankers, February 5, showed very few spores and these failed to germinate. Of 46 pycnidia examined March 14, 35 bore no spores, 8 contained very few, and 3 were nearly filled with them. No germination was obtained. On May 9, of 45 pycnidia examined one contained no spores, 9 very few, 19 many, and 16 were full of them. Germination of a few spores occurred in 4 days, 54 per cent in 5 days, and over 60 per cent in 6 days.

In this connection some unpublished observations made in this laboratory by Mix may be cited. Pycnospores were found in bark cankers on February 15 and March 7 and a few spores germinated in distilled water. Spores from pycnidia on twigs were put to germinate, on May 24, by the slide and drop method. Of six slides examined after 18 hours, only one gave a percentage germination and that was 45 per cent. On June 5, the average germination of spores from twig-cankers was 9 per cent in contrast to 90 per cent for spores from culture. On July 18, abundant germination was obtained of spores from cankers.

TABLE 4.—*Effect of different ages on germination of spores of Phyllosticta solitaria from culture*

| Time after appearance of spores | Culture number | Germination | | Average length of germ-tubes | |
|---|---|---|---|---|---|
| *Days* | | *Per cent* | | *Microns* | |
| After | | 48 hrs. | 96 hrs. | 48 hrs. | 96 hrs. |
| 1 | 24a | 0 | 0 | 0.00 | 0.00 |
| | 342 | 2 | 4 | 0.28 | 0.35 |
| | 299 | ..... | 67 | ..... | 86.22 |
| | 24b | ..... | 4 | ..... | 0.40 |
| | 24c | 0 | 0 | 0.00 | 0.00 |
| 2 | 24a | 26 | 30 | 2.32 | 3.22 |
| 3 | 342 | 10 | 12 | 0.93 | 1.37 |
| | 24c | 0 | 2 | 0.00 | 0.08 |
| 4 | 24a | 22 | 32 | 3.45 | 5.16 |
| | 24b | ..... | 31 | ..... | 3.02 |
| 5 | 24a | 30 | 42 | 3.81 | 4.34 |
| | 342 | 11 | 12 | 1.02 | 1.33 |
| | 299 | ..... | 88 | ..... | 36.44 |
| | 24c | ..... | 8 | ..... | 0.89 |
| 7 | 24a | 50 | 57 | 5.20 | 9.14 |
| | 342 | 12 | 14 | 1.15 | 1.14 |
| | 24c | 30 | 34 | 1.73 | 1.88 |
| 8 | 299 | 93 | 93 | 34.28 | 31.80 |
| | 24b | 53 | 59 | 9.64 | 12.54 |
| 11 | 24d | 16 | 16 | 3.24 | 3.19 |
| | 342 | ..... | 34 | ..... | 4.48 |
| | 299 | ..... | 95 | ..... | 36.05 |
| | 24b | ..... | 63 | ..... | 12.76 |
| | 24c | ..... | 36 | ..... | 2.50 |
| 15 | 24d | ..... | 22 | ..... | 2.22 |
| | 342 | 35 | 40 | 5.34 | 5.10 |
| | 299 | 95 | 97 | 35.96 | 36.74 |
| | 24b | 22 | 22 | 1.42 | 1.99 |
| | 24c | ..... | 22 | ..... | 1.23 |
| 17 | 299 | 97 | ..... | 25.73 | ..... |
| | 24b | 22 | ..... | 1.83 | ..... |
| 19 | 24d | 53 | 62 | 6.51 | 8.57 |
| | 342 | ..... | 10 | ..... | 1.34 |
| | 299 | ..... | 98 | ..... | 32.98 |
| | 24c | 16 | 16 | 1.13 | 1.20 |
| 22 | 299 | 97 | 98 | 38.07 | 38.78 |
| | 24b | 9 | 9 | 1.41 | 1.08 |
| | 24c | 16 | 16 | 0.96 | 1.12 |
| 25 | 24d | 60 | 69 | 8.38 | 9.61 |
| | 342 | ..... | 6 | ..... | 1.09 |
| | 24c | ..... | 9 | ..... | 1.04 |
| 29 | 342 | ..... | 4 | ..... | 0.43 |
| | 24b | ..... | 8 | ..... | 0.77 |

Crosier, also working in this laboratory, found a few spores present in twig cankers on February 10, and obtained 13 per cent germination in bark decoction and 3 per cent in tap water in 24 hours.

The above data and the observations of other workers here cited indicate a longer interval between morphological and physiological maturity for spores from twig-cankers than for spores from culture. It is possible that a closer agreement might be found between spores formed in fruit blotches and those formed in culture.

### EFFECT OF VOLATILE SUBSTANCES ARISING FROM PLANT TISSUES

The effect of various plant tissues on germination when placed near the hanging drop was observed. Apple-fruit tissue, apple bark, elm bark, pear bark, potato tuber, orange pulp, orange rind, onion scale, and onion leaf were used. Small pieces (approximately $1 \times 6 \times 6.5$ mm.) and the larger pieces ($1 \times 9 \times 10$ mm.), of each tissue were placed in the bottom of the cell, a hanging drop of distilled water containing spores being immediately above but not touching the plant tissue. The slides were placed in Petri-dish moist chambers. Spores were counted after 40 hours, since bacterial contamination could not be entirely prevented and counts made on the 3rd and 4th days would not have been reliable. In each case spores in distilled water without pieces of plant tissue served as a control.

The results of this study are shown in table 5. Bark tissue stimulates germination, apple bark being the most effective. On the other hand apple-fruit tissue, potato tuber, onion leaves and scales, and orange rind and pulp inhibit germination. Brown (2), in his studies on the germination of spores of *Botrytis cinerea* when exposed to volatile substances arising from plant tissues, found germination reduced or inhibited by potato tuber and onion leaves or bulb scales. He demonstrated that carbon dioxide was not the inhibiting factor. In the present investigation no experiments were attempted to find what might be the inhibiting or stimulating factor involved.

### SUMMARY

Studies were made of the effect of various factors on the germination of spores of *Phyllosticta solitaria*.

Spores were taken, for the most part, from culture, suspended in distilled water, and germination counts were made at the end of 48 hours.

Light has no appreciable effect on germination.

The minimum temperature for germination is somewhat below 5° C., the maximum somewhat above 39° C., the optimum is close to 23° C.

Spores from pycnidia in bark cankers were found to have the same temperature requirements as spores from culture, but showed consistently lower germination. This may have been due to physiological immaturity of the spores from bark cankers.

TABLE 5.—*Effect of various plant tissues placed in the germination chamber on germination of spores of Phyllosticta solitaria from culture*

| Tissue | Trial number | Germination | | Average length of germ tubes | |
|---|---|---|---|---|---|
| | | *Per cent* | | *Microns* | |
| | | small piece | large piece | small piece | large piece |
| Control—distilled water | 1 | 12 | 12 | 0.86 | 0.86 |
| | 2 | 52 | 52 | 10.77 | 10.77 |
| Apple-fruit tissue | 1 | 0 | 0 | 0.00 | 0.00 |
| | 2 | 23 | 13 | 2.55 | 1.58 |
| Apple bark | 1 | 11 | 26 | 0.64 | 2.34 |
| | 2 | 69 | 72 | 23.76 | 25.10 |
| Elm bark | 1 | 10 | 11 | 0.97 | 1.06 |
| | 2 | 35 | 59 | 7.35 | 10.18 |
| Pear bark | 1 | 11 | 25 | 1.10 | 1.86 |
| | 2 | 52 | 53 | 11.12 | 10.98 |
| Potato-tuber tissue | 1 | 0 | 2 | 0.00 | 0.04 |
| | 2 | 42 | 51 | 2.73 | 5.88 |
| Orange-fruit tissue | 1 | 0 | 2 | 0.00 | 0.05 |
| | 2 | 2 | 3 | 0.16 | 0.26 |
| Orange rind | 1 | 0 | 0 | 0.00 | 0.00 |
| | 2 | 6 | 7 | 1.68 | 1.12 |
| Onion scale | 1 | 0 | 0 | 0.00 | 0.00 |
| | 2 | 1 | 0 | 0.08 | 0.00 |
| Onion leaf | 1 | 0 | 0 | 0.00 | 0.00 |
| | 2 | 4 | 2 | 0.57 | 0.52 |

When, instead of percentage germination, average germ-tube length (taking into account both germinated and nongerminated spores) was taken as the measure of viability, the results agreed in nearly every case with those obtained by the former method.

The observed temperature requirements for spore germination of *Phyllosticta solitaria* do not help to explain the present distribution of the fungus in nature.

Spore germination was more profuse in apple-bark decoction and potato-dextrose broth than in distilled water. Coons' solution caused a slight increase; tap water, rain water, apple-fruit juice, and K. U. solution had an inhibitory effect.

Spores of *Phyllosticta solitaria* formed in culture are capable of immediate germination; but increasingly better germination occurs with age, the maximum occurring when the spores are ten days or two weeks old. There is thus a brief interval between morphological and physiological maturity of spores formed in culture.

Of various tissues placed in the hanging drop, bark tissue acted as a stimulus, apple bark being the most effective. Orange tissue (both rind and fruit), onion scale and leaf, and apple pulp were somewhat inhibitory. No attempt was made to identify the volatile inhibiting substances.

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# THE GLADIOLUS DRY ROT CAUSED BY SCLEROTINIA GLADIOLI (MASSEY) N. COMB.[1, 2]

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(Accepted for publication June 29, 1933)

This disease is of importance in the growing of gladioli in commercial plantations as well as in private gardens. It has received some attention from the pathological point of view and its causal fungus has been studied and described (1, 6). The prominent structure of the fungus is a minute sclerotium, and the failure to find any functional spores, either on diseased plants or in artificial cultures, made it necessary to designate it as one of the Mycelia Sterilia under the binomial *Sclerotium gladioli* Massey (6).

While working with several sclerotium-producing fungi, it was found that this fungus will develop fruiting bodies as the result of a sexual process. The microconidia noted by Massey, and an ascogonial system in receptive structures, comprise the sexual components that accomplish the formation of apothecia of the Sclerotinia type. The recognition of a perfect stage makes it possible to extend our knowledge of this pathogen, and the purpose of this paper is to propose a new combination for its designation and to record the emended diagnosis. A number of new suscepts have been encountered in the course of this work. These will be mentioned, together with some historical facts relative to the disease and the pathogen.

## THE SEXUAL NATURE OF THE FUNGUS

The fact that fruiting bodies of this fungus develop as the result of a sexual process has already been reported in a brief preliminary paper (2). A description of the technique used to induce the development of apothecia, together with other facts relating to the sexual mechanism of this fungus, has been published in a separate paper (3). It may not be out of place, however, to refer to some of these points here.

The microconidia of *Sclerotinia gladioli* are similar in shape and manner of production to those that have been recorded in several species of Sclero-

[1] Contribution No. 123 from the Laboratories of Cryptogamic Botany, Harvard University.

[2] Part of this work is taken from a thesis submitted to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy and the rest was done during the tenure of a National Research Fellowship in the Biological Sciences at the Laboratories of Cryptogamic Botany, Harvard University.

[3] The generous assistance and stimulating encouragement of Professor H. H. Whetzel, Professor W. H. Weston, Jr., and Dr. D. H. Linder are gratefully acknowledged.

tinia, Botrytis, and Sclerotium. Their function hitherto has always been obscure, but this investigation established the fact that they are spermatia. The receptive structures containing the coiled ascogonial system arise from a stromatic layer formed on the surface of substrates rich in carbohydrates. The spermatization of these with microconidia from a compatible isolate of the fungus results in the development of apothecia. In the absence of spermatia, or if microconidia from the same or an incompatible isolate are used for spermatization, there may be some elongation of the receptive bodies but no development of apothecia. Each monomycelial or monoascosporic culture gives rise to both receptive bodies and microconidial sporodochia when subjected to favorable conditions, but such cultures are self-sterile, reciprocally intersterile with some isolates (incompatibles), and interfertile with others (compatibles). There exists, therefore, the phenomenon of monoecism, if one may use this term to designate the presence of both sexual organs on the same fungus thallus, with the separation of various isolates into 2 groups exhibiting reciprocal intragroup sterility, intergroup fertility, while each isolate itself is self-sterile.

## THE DISEASE AND THE PATHOGEN

*Sclerotinia gladioli* is responsible for a necrotic disease, known as dry rot, of the gladiolus and certain allied plants. The outstanding symptom is premature death brought about by the invasion and decay of the subterranean organs, including the basal portion of the leaf sheaths, while on the corms and cormels characteristic lesions are produced, often accompanied by vascular necrosis and mummification. On the decayed parts of the leaf sheaths, on the corm scales, and sometimes in or on the corm lesions are formed minute sclerotia, which serve as organs of resistance. The stroma, referred to above, is formed within badly diseased corms.

The first work of any importance done on this disease was by Wallace,[4] who applied the name dry rot to one of the 4 diseases of gladiolus corms that he studied. He suspected that he was including 2 diseases under this name, and, judging from his descriptions and photographs, this was the case. In addition to the true dry rot, he apparently had material of a common but undescribed storage disease of the corms caused by *Botrytis* sp. His description of one of the fungi isolated from gladiolus corms from Germany and New York State is unquestionably that of *Sclerotinia gladioli*. Two years later, Fitzpatrick (4) restricted the use of the name dry rot to the disease as now recognized.

Even before 1909, however, this disease seems to have been seen, although not recognized as such, for Wallace thinks that this was probably

[4] Wallace, E. Some bulb rots of gladioli. Unpublished thesis. Cornell University, 1909.

the disease referred to by Robinson (8, p. 139) in 1883 in England. Yet Wallace and other investigators have overlooked an article in 1906 by Ritzema-Bos (7) in which he describes a disease occurring on gladiolus and montbretia plants in Sassenheim, Holland. It is quite clear that his material consisted of plants affected with dry rot.

These several investigators all observed the development of small sclerotia by the causal fungus and noted the absence of sporulation. Hence, in 1918, Massey (5) proposed that it be placed in the form genus Sclerotium, and subsequently the present writer (1) referred to it as *Sclerotium* sp. in 1926. In 1928 Massey (6) erected the binomial *Sclerotium gladioli* and, as he observed for the first time the presence of microconidia, he suggested that the fungus might be a Sclerotinia because of the structure of these bodies and of the sclerotium, even though his attempts to bring about the development of apothecia were unsuccessful. These microconidia were found by Massey in 25- to 40-day-old test-tube cultures, where they appeared as small white granules less than 1 mm. in diameter, buried in the medium at the back of the slant adjacent to the wall of the tube. The writer finds, however, that by using Petri-dish cultures composed of plant stems and potato–2 per cent dextrose agar it is now possible to obtain more numerous and larger microconidial sporodochia in 12 to 18 days.

All of the papers dealing with this disease hitherto have noted its occurrence on the several varieties of the large-flowering gladiolus included in hybrids of the species *G. primulinus, G. communis,* etc. In the course of the present investigation, however, certain additional suscepts have been encountered among other genera of the Iridaceae. During a visit to Holland in 1928, an examination of crocus corms as they were brought in from the field revealed the presence of this disease in a great many cases. In the same locality, also, under greenhouse culture, it was found on plants of *Lapeirousia* (*Anomatheca*) *cruenta* Baker and in field culture on *Tritonia* (*Montbretia*) *crocosmaeflora* Lemoine. Rather severe infections were observed on freesia plants in several greenhouses in Long Island, New York, in 1929, and in shipments of corms of this plant from Southern Europe to Canada. In all of these instances, the causal fungus was isolated and its identity confirmed. In connection with the question of host ranges, it is of interest to note here that when several varieties of rhizomatous iris, tulip, hyacinth, and narcissus were planted by the writer in soil from which gladiolus plants exhibiting 100 per cent infection had just been harvested, these plants all grew well and never showed any sign of infection. These tests, although limited, were sufficiently conclusive to justify mentioning them here, for a knowledge of the range of resistance and susceptibility among the commoner bulbous and rhizomatous ornamental plants may be

of importance to the nurseryman and gardener in arranging the rotation of their plantings.

EMENDED DIAGNOSIS

The recognition as sexual elements of the microconidia and the receptive structures developed by the stroma, and the manipulation of these with resulting production of a fruiting body, now makes it possible to give a complete technical description of this fungus, as follows:

**Sclerotinia gladioli** (Massey) n. comb.

Synonymy—*Sclerotium gladioli* Massey L. M. Phytopath. **18**: 519–529. 1928.

Mycelium septate, inter- and intracellular, multinucleate; in culture white, aerial portions tipped with buff when old, emitting a musty odor.

Sclerotia black, 90–240 x 90–300 μ, average 191 x 164 μ, consisting of a well-defined and compact rind of thick-walled cells surrounding a thin-walled, pseudoparenchymatous medulla, the cells of which are filled with small globules of oil (Fig. 1, A).

Stromatic tissue formed on substrates rich in carbohydrates, at temperatures of 18° to 24° C. Usually firmly attached to the substrate. Black, varying in thickness from 80–500 μ, with a rind of loosely aggregated, closely septate, black, thick-walled hyphae, and a prosenchymatous, hyaline medulla (Fig. 1, A).

Receptive bodies formed from the stromatic layer, .8 to 1.9 mm. tall, depending on age, .4 to .8 mm. broad, columnar, often branched, tapering to a rounded apex or occasionally slightly capitate, light brown, pilose, and covered with a thin layer of a mucilaginous substance. These bodies possess an external region of loosely interwoven, thick-walled, septate hyphae, hyaline towards the apex, becoming progressively darker towards the base and merging with the black hyphae of the stromatic rind. Within this, a region of light brown, densely packed hyphae, somewhat interwoven but running longitudinally and giving rise to an apical tuft of thinner-walled, septate hyphae that arch inwards to form a depression at the center of the apex. In the center is found a column of hyaline, less compact tissue composed mainly of a sparsely-septate, intricately coiled, multinucleate ascogonial system, which is terminated at the apex by trichogynous hyphae with their tips clustered beneath the overarching apical hyphae. On fertilization these structures develop into apothecia (Fig. 1, A, B, D).

Microconidia globose, 1.2–1.8 μ in diameter, uninucleate, and incapable of germination, but functioning as spermatia. Produced in a sporodochium made up of closely septate hyphae that give rise to numerous clusters of verticillately branched conidiophores that end in tapering, elongate, slightly

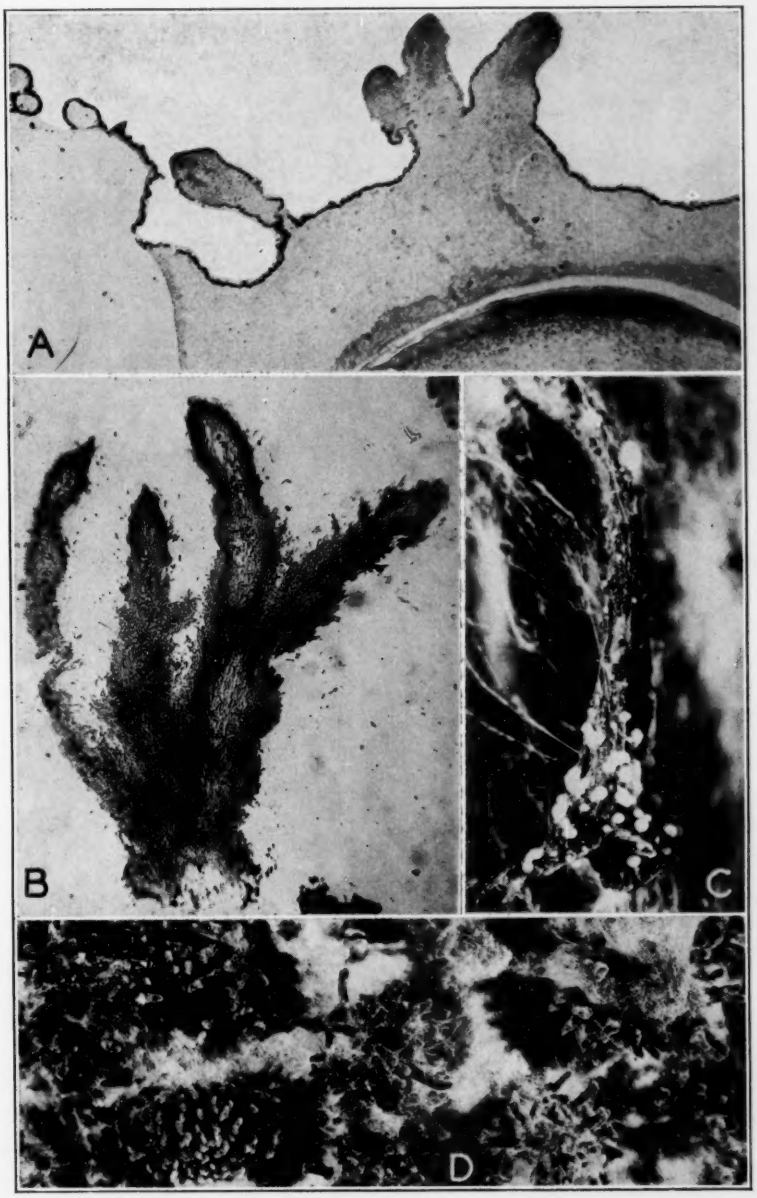


FIG. 1. A. Receptive bodies arising from the stroma formed on grain of wheat, a portion of which is evident at the lower right. Note the thin loosely constructed rind of the stroma as compared with the thicker and more compact rind of the three sclerotia at the upper left. ×30. B. Branched receptive body showing the coiled ascogonial hyphae in the central core. ×35. C. Microconidial sporodochia. ×4.5. D. Part of a Petri-dish culture with receptive bodies. ×4.5.

bent terminal cells on which the microconidia are developed successively in vast numbers embedded in a mucilaginous matrix, which, on drying, gives a waxy consistency to the whole sporodochium (Figs. 1, C and 2, C).

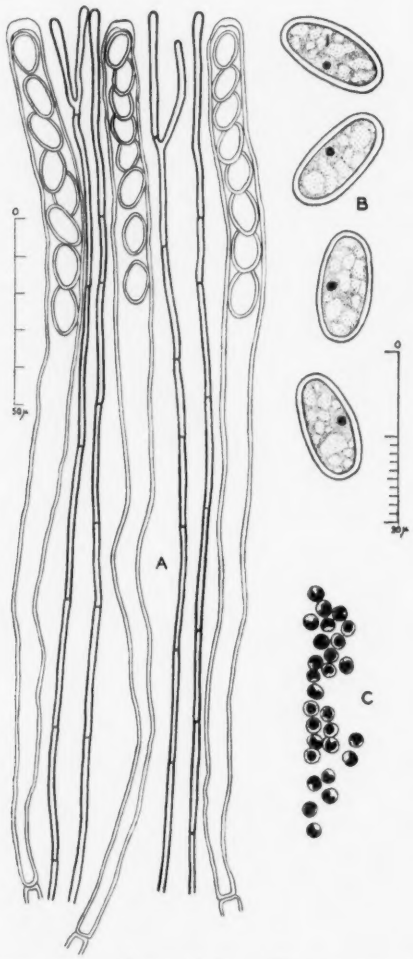


FIG. 2. A. Asci, ascospores, and paraphyses. B. Ascospores more highly magnified. C. Microconidia, magnified to the same extent as the ascospores.

Apothecia densely cespitose, stipitate, 3 to 7 mm. broad, 6 to 10 mm. high. Disc cinnamon brown, stipe chestnut brown (Ridgway). Hymenial surface umbilicate, convex-discoid. Margin strongly reflexed, deeply-crenate, sometimes entire or convolute. Lower surface tomentose, less densely so down the stipe. Context thick, prosenchymatous, infundibuliform, with a definite hypothecium (Fig. 3A, B, and C). Asci cylindrical to cylindroclavate, opening by a pore, 190.5–235.4 x 8.5–9.2 μ, average 212.5 x 9.06 μ. Ascospores 8, unicellular, uniseriate, ellipsoidal, hyaline, uninucleate, 10.2–

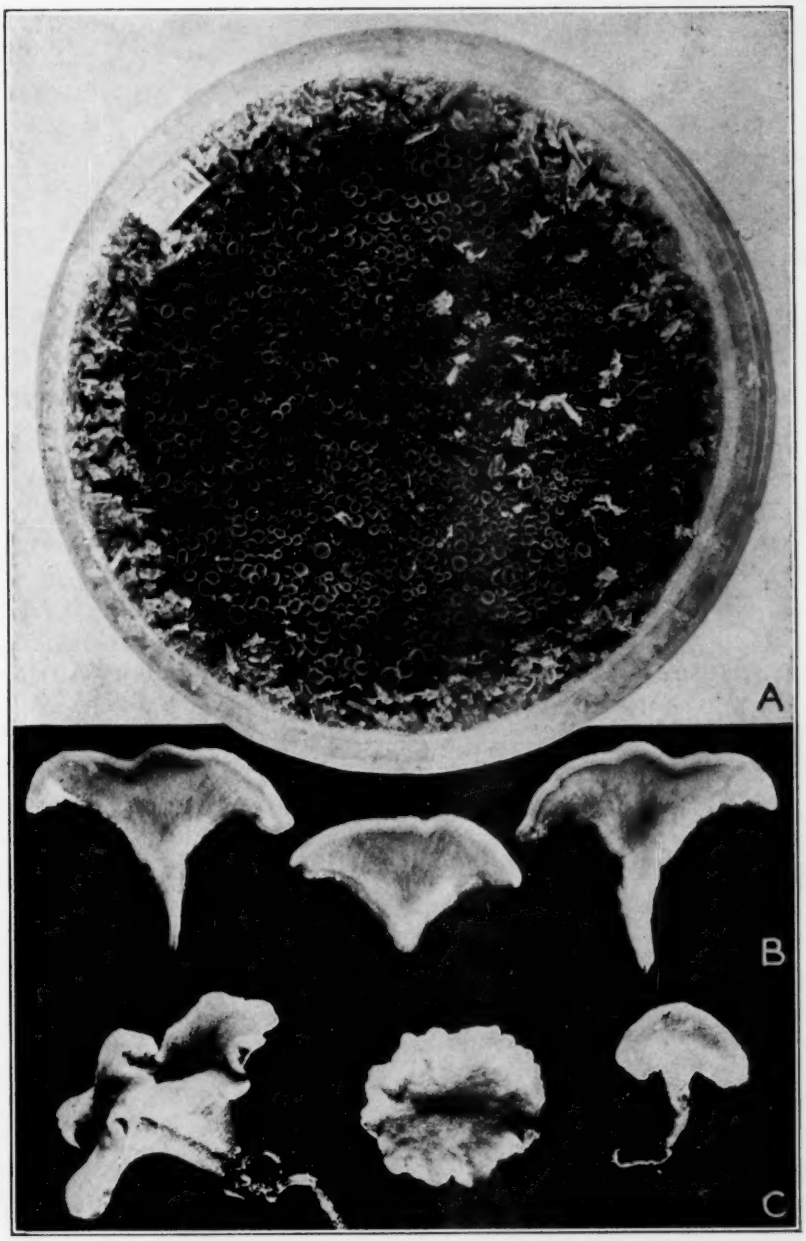


FIG. 3. A. Petri-dish culture bearing numerous apothecia. ×.8. B. Longitudinal sections of apothecia. Note hypothecium, thick medullary excipulum, and tomentose excipulum. ×3.4. C. Three apothecia showing variation in shape. ×4.2.

16.75 x 5.6–9.5 μ, average 14.04 x 7.25 μ, mode 13.5 x 7.25 μ. Paraphyses abundant, filiform to slightly clavate at apex, septate, hyaline 2.8–3.2 μ diam. (Fig. 2, A and B).

The cause of a necrotic disease known as dry rot, on the following suscepts:—All cultivated varieties of *Gladiolus* spp., *Tritonia* (*Montbretia*) *crocosmaeflora* Lemoine, *Freesia* spp., *Lapeirousia* (*Anomatheca*) *cruenta* Baker, and *Crocus* spp.

Known distribution—United States, Canada, England, Scotland, Ireland, Holland, Germany, France, and New Zealand.

Type specimens of apothecia deposited in the Plant Pathology Herbarium, Cornell University, Ithaca, N. Y. No. 20136. Also in the Farlow Herbarium, Harvard University, Cambridge, Mass.

## SUMMARY

The dry-rot disease of gladioli has been known for several years and its importance appreciated. The causal fungus apparently developed no functional spores and hence was named *Sclerotium gladioli* by Massey in 1928.

A sexual mechanism has now been discovered that involves the microconidia and the pilose receptive structures developed from a stroma. The sexual interaction of these yields apothecia of the Sclerotinia type. The life cycle of the fungus is, therefore, now completely known and its taxonomic relationship can be established.

The historical events of importance in connection with this disease and its pathogen are given.

The resistance and susceptibility of several common ornamental plants are mentioned and a member of new suscepts are recorded.

The new combination *Sclerotinia gladioli* (Massey) is proposed and a detailed emended diagnosis is given.

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# HOST SPECIALIZATION OF PUCCINIA SORGHI

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(Accepted for publication July 8, 1933)

Arthur (2) was the first to produce the aecial stage of the rust of maize, *Puccinia Sorghi* Schw. In Indiana he found aecia on *Oxalis cymosa* in close association with overwintered débris of maize bearing telia of the rust. Aeciospores sown on young plants of maize resulted in the production of uredinia. The following year the connection was verified by inoculating *Oxalis cymosa, O. corniculata, O. Ortgiesii, O. Bowiei,* and *O.* sp. with over wintered teliospores (Arthur, 4). Pycnia and aecia developed only on *O. cymosa.* Aecispores from the infection on Oxalis, sown on young maize plants, produced uredinia. Arthur was not able to infect maize from germinating teliospores, as Kellerman (18) had reported. Kellerman's results (19) were probably due to a mixture of viable urediniospores with the teliospores used in his experiments. Urediniospores may survive the winter to some extent. The writer has been able to obtain germination of urediniospores in late March from maize leaves that had been partly protected in the field (through the winter) in shocks of maize.

Kellerman, later, (19) also procured aecia on Oxalis from teliospores. In Iowa, Smith (25) obtained aecia on *O. corniculata. O. europea,* and *O. tropaeoloides.* Only pycnia were produced on *O. valdiviensis.* Negative results occurred with *O. cernua* and *O. rubra.* Miss Rice (24) obtained aecia on "*Xanthoxalis stricta* (L.) Small" and "*X. corniculata* (L.) Small" from inoculations with teliospores collected at Ann Arbor, Michigan.

In South Africa, Mary Pole Evans (16) found that *Oxalis corniculata* was a susceptible host for the aecial stage. *O. tenuifolia, O. livida, O. lateriflora, O. variabilis, O. balsamifera, O. monophylla,* and *O. hirta* were immune. In Austria, Hecke (17) inoculated a series of species of Oxalis with teliospores of *Puccinia Sorghi.* He obtained an abundant development of aecia on *O. stricta. Oxalis trapaeoloides* was less susceptible, and the aecia took six days longer to develop. Only pycnia were produced on *O. rosea.* Only flecks showed on *O. valdiviana; O. Bowiei* gave negative results.

[1] The investigations upon which this study is based were made while the writer was a member of the staff of the Botanical Department of the Purdue University Agricultural Experiment Station and of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. Papers from the Department of Botany and the Herbarium of the University of Michigan, No. 410.

Tranzschel (27) produced aecia on *O. corniculata* from telia collected in Transcaucasia.

Long (20) found aecia on *Oxalis corniculata* closely associated with rust on *Andropogon furcatus* at Denton, Texas. Telia were used to inoculate *O. corniculata,* and infection was obtained, but the plants were accidentally killed before aecia developed. Arthur and Fromme (8, p. 278) have assigned this rust to *Puccinia Sorghi.*

According to Arthur and Fromme, aecia of *Puccinia Sorghi* have been collected in North American on *Ionoxalis violacea* (L.) Small (*Oxalis violacea* L.), *Xanthoxalis filipes* Small (*Oxalis filipes* Small), *X. Langloisii* Small, and *X. stricta* (L.) Small (*Oxalis stricta* L., *O. corniculata stricta* Sav., *O. cymosa* Small). In their monograph the Sydows (26) list *Oxalis Bowiei, O. corniculata, O. filipes, O. Langloisii, O. obtusa, O. rosea, O. stricta,* and *O. violacea* as hosts.

Cummins (12) reports studies from which he concludes that *Puccinia Sorghi* is heterothallic. He obtained formation of aecia in 75 per cent of isolated infections treated with pycnial exudate from other infections. Of those left untreated only 14.3 per cent formed aecia.

The identities of some of the species of Oxalis are somewhat difficult to determine. Wiegand (28) has discussed the confusion that exists among the yellow-flowered species. He places *Xanthoxalis filipes* under *Oxalis filipes, X. Langloisii* under *O. corniculata, X. stricta* under *O. stricta,* and *O. corniculata stricta* and *O. cymosa* under *O. europea.*

For these investigations seeds of a number of species of Oxalis were obtained from various botanical gardens. Several of the local species were sent to Professor Wiegand for identification, and his determinations have been followed in naming the plants of *Oxalis corniculata, O. europea,* and *O. stricta.*

During the spring of 1928 a number of plants of *Oxalis corniculata* were infected in a manner similar to that used by Craigie (9, 10, 11). Leaves of maize bearing germinable teliospores were placed at different heights over plants of Oxalis in a moist chamber and were left for various periods until a combination was found that gave such a scattering of basidiospores that a large number of monosporous infections resulted. After inoculation the plants were placed under muslin cages to protect them against insects and were watered from below to prevent the spread of pycniospores by spattering. As soon as the first signs of infection (indicated by slight flecks) were noted all leaves bearing more than one infection were removed. With a little experience it was not difficult to detect infections occurring close together.

After the isolated spots had produced pycnia and after pycniospores

were exuding, a mixture of the exudate from a number of such spots was made. This was transferred to a series of isolated groups of pycnia. Another series was left untreated. In the one treated with the mixed exudate 260 out of 290 monosporous infections produced aecia. Of the 342 in the untreated series, only 29 produced aecia and 313 remained undeveloped.

Aecia were produced even if the transfer of pycniospores was delayed for some time. Thus in a series of plants of Oxalis inoculated on February 20, 71 isolated infections were treated with mixed exudate on March 4 and 147 were left untreated. In the treated group aecia were produced by 69 infections and in the untreated by only 11. Of those that did not produce aecia, 86 were treated with mixed exudate on March 25, 79 of which developed aecia. Thus the thalli were still in a condition favorable for diploidization 34 days after infection. These results agree with those reported by Cummins (12). They indicate that *Puccinia sorghi* is self-sterile.

From 1925 to 1928 an attempt was made to throw additional light on the host range of the aecial stage. The results are given in the accompanying table. In addition to testing the species of Oxalis listed in the table several collections of telia were sown on *Baptisia australis* (L.) R. Br., *Polygala Senega* L., *Pentstemon laevigatus* Ait., *P. secundiflorus* Benth., and *Viola papilionacea* Pursh, with negative results.

*Oxalis corniculata* was found to be the most favorable host, being susceptible to all collections of telia and producing an abundance of aecia. *O. stricta* gave mostly negative results; occasionally pycnia, and in one instance a few aecia, were produced. *Oxalis cernua* gave somewhat similar results. *O. bipartita* produced pycnia accompanied by necrosis in one case; the other results were negative. With the exception of *O. europea,* all the other species of Oxalis gave negative results. *O. europea* presented an interesting situation. To collections 2 and 5 it was susceptible, and abundant aecia[2] were produced. To the other collections it was resistant, at the most producing only a few pycnia.

It is evident that *Puccinia Sorghi* can be divided into at least 2 races: one producing only a few pycnia on *Oxalis europea;* the other, abundant aecia. It may be that Arthur (4) dealt with the latter, since he obtained aecia on *O. cymosa* (= *O. europea,* according to Wiegand). However, Arthur was not able to infect *O. corniculata,* whereas in this study both the collections of telia which infected *O. europea* also infected *O. corniculata.* These collections may, however, have been mixtures of two races: one producing aecia on *O. corniculata,* not on *O. europea;* the other, aecia on

[2] Collection 5 was received from M. A. Smith, and the results obtained agree with those reported by him (25).

*O. europea*, not on *O. corniculata*. If they are not mixtures, the existence of a third race is indicated, distinguished by having both *O. corniculata* and *O. europea* as susceptible species. Still another race may be indicated by the results of Hecke (17) and Rice (24), who obtained aecia on *O. stricta*. This species of Oxalis, as interpreted by Wiegand, was resistant to all telial collections used in this study. Aecia collected on *O. filipes* and *O. violacea* have also been assigned (Arthur and Fromme, 8) to *Puccinia Sorghi*. Neither of these species proved to be susceptible in these investigations.

TABLE 1.—*Relative susceptibility of species of Oxalis to Puccinia Sorghi*

| Species inoculated | Reaction to collections of telia[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1[b] | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Oxalis articulata Sav. | | | | | | – | – | |
| " bipartita A. St. Hil. | | | – | | O | – | | |
| " brasiliensis Lodd. | – | – | | | – | – | – | |
| " carnosa Mol. | – | – | | | – | – | – | |
| " cernua Thunb. | – | – | | | O(I) | – | – | |
| " corniculata L. | OI | OI | OI | OI | OI | OI | OI | OI |
| " crassipes Urb. | – | – | | | – | – | – | |
| " Deppei Lodd. | – | – | – | | – | – | | |
| " europea Jord. | – | OI | –(O) | –(O) | OI | – | – | – |
| " filipes Small | | | | | | – | – | |
| " floribunda Lehm. | | | | | | – | – | |
| " lasiandra Zucc. | – | – | – | | – | – | | |
| " latifolia H. B. & K. | – | – | – | | – | – | – | |
| " stricta L. | – | – | –O | | – | – | – | O(I) |
| " valdiviensis Barn. | | – | | | O | – | – | |
| " violacea L. | – | – | – | | – | – | – | |

[a] – = negative results
(O) = few pycnia
(I) = aecia produced in abundance
O = pycnia produced in abundance
I = aecia produced in abundance

[b] Collections of telia were made as follows: 1–2, at La Fayette, Indiana (studied in 1925); 3–4, at La Fayette (studied in 1926); 5, in Decatur Co., Iowa, by M. A. Smith (studied in 1926); 6–7, at La Fayette (studied in 1927); and 8, at La Fayette (studied in 1928).

As previously shown (Mains, 22, 23), several physiologic forms may be distinguished in *Puccinia Sorghi* by the reactions of a selected set of inbred lines of maize. The relationship of these physiologic forms to the races differentiated by *Oxalis europea* has not been throughly established. Of those collections to which *O. europea* was resistant, collection 3[3] proved to

[3] Aeciospores produced by the infection of Oxalis were used to inoculate a series of differential strains of maize.

be physiologic form 1; collection 1, a mixture of physiologic forms 1 and 3; and collections 6 and 8, physiologic form 4. Collection 2, to which *O. europea* was susceptible, proved to be physiologic form 3. These results do not indicate any correlation, but they are not extensive enough to justify conclusions.

In 1920 aecia produced on *Oxalis corniculata* were used to inoculate seedlings of *Andropogon furcatus, A. scoparius,* and *Zea mays* (Golden Bantam). Only maize developed uredinia. In 1921 aecia from *Oxalis corniculata* were used to inoculate seedlings of *Andropogon furcatus* Muhl., *A. scoparius* Michx., *Holcus Sorghum* L., *Tripsacum dactyloides* L., *Sorghastrum nutans* (L.) Nash, *Coix lachryma-jobi* L., *Euchlaena mexicana* Schrad. (*Florida teosinte*), and *Zea mays* (Golden Bantam). Only teosinte and maize showed infection, both developing abundant uredinia.

The results obtained from aecia were supported by those secured from uredinia. Rust collected on maize at La Fayette, Indiana, in 1920 and sown on seedlings of *Andropogon furcatus, A. scoparius,* and *Zea mays* resulted in the development of uredinia on maize only. Likewise, in 1922, inoculations with urediniospores collected at La Fayette, Indiana, produced uredinia on maize. *A. furcatus, A. scoparius, Erianthus divaricatus* (L.) Hitchc., *Coix lachryma-jobi,* and *Tripsacum dactyloides* gave negative results. These results agree with those of Kellerman (18), who was able to infect teosinte "*Euchlaena luxurians,*" but not *Sorghum vulgare, Saccharum officinarum,* or *Tripsacum dactyloides.*

Arthur and Fromme (8) list *Andropogon furcatus* as a host for *Puccinia Sorghi.* The results obtained in these investigations indicate that the rust on maize probably does not infect Andropogon and is at least a distinct race. It seems doubtful whether the rust on *A. furcatus* belongs in *Puccinia Sorghi.* This disposition of it was apparently based on cultures by Long (20), who showed that rust on *A. furcatus* produced aecia on *Oxalis corniculata.* Previous investigations have shown that rust on Andropogon (*Puccinia Ellisiana, P. Andropogonis*) produces aecia on species of Viola (Arthur, 7; Long, 20, 21), Pentstemon (Arthur, 1, 5, 6; Long, 21), and Comandra (Arthur, 3, 4). Davis (13, 14, 15) has found that *P. Andropogonis* also develops aecia on species of Polygala, Lupinus, Amphicarpa, and Xanthoxylum. Apparently *P. Andropogonis* contains a number of races differing in their aecial hosts. It would seem best, therefore, to consider the Andropogon-Oxalis rust another race of *P. Andropogonis,* rather that a race of *P. Sorghi.*

## SUMMARY

1. Additional evidence is given that *Puccinia Sorghi* is self-sterile. Aecia do not usually develop from infections from single basidiospores.

2. Pycnia and aecia were produced on *Oxalis corniculata* and *O. europea*. Pycnia and occasionally a few aecia developed on *O. cernua* and *O. stricta*. Pycnia rarely developed on *O. bipartita* and *O. valdiviensis*. Negative results were obtained with *O. articulata, O. brasiliensis, O. carnosa, O. crassipes, O. Deppei, O. filipes, O. floribunda, O. lasiandra, O. latifolia,* and *O. violacea*.

3. *Oxalis corniculata* was very susceptible to all the collections of the rust that were studied. Aecia were obtained on *O. europea* with only 2 out of the 8 collections. This indicates that there are 2 races of *Puccinia Sorghi* that may be distinguished by the susceptibility of *O. europea*.

4. Since aecia have been collected on *Oxalis filipes, O. stricta,* and *O. violacea,* the marked resistance of these species noted in these investigations indicates other races of *Puccinia Sorghi*.

5. Inoculations from rust derived from maize (aecia and uredinia) produced uredinia on maize and teosinte. Negative results were obtained with *Andropogon furcatus, A. scoparius, Coix lachryma-jobi, Erianthus divaricatus, Holcus Sorghum, Tripsacum dactyloides,* and *Sorghastrum nutans*.

6. It is suggested that the Andropogon rust with aecia on species of Oxalis, which has been placed in *Puccinia Sorghi*, should be considered a race of *Puccinia Andropogonis*.

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# A PHYTOPHTHORA DISEASE OF SNAPDRAGONS

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In March, 1932, a wilt of snapdragons was found in several greenhouses near San Leandro, California. The disease was first found in the flats into which the young plants were transplanted from the seed bed. When the grower further transplanted the snapdragon plants to the large beds in the greenhouse, he selected apparently healthy specimens from flats on which some damage had been done by the disease. Later the disease appeared in the large greenhouse beds, and eventually 50 per cent of the plants were affected. Injury ranged from killing soon after transplanting to wilting when nearly ready to produce flowers. The grower had not experienced the trouble prior to this time and was unable to discover the source of the disease. A number of the affected plants were sent by him to the plant pathological laboratory of the California State Department of Agriculture, where the cause of the wilt was determined.

## SYMPTOMS OF THE DISEASE

Wilting of the plants was brought about by a girdling of the stems at the ground line or slightly above it. A slightly yellow water-soaked area appeared on the healthy white stem tissue. As this lesion increased in size the older portions of the lesion became yellow, then brown, and finally almost black. The lesion usually enlarged until it girdled the plant and extended up and down the stem for several inches. In the last stages of the disease the outer portion of the stem sloughed away, exposing the hard woody xylem tissue.

Plants when first attacked wilted slightly during the middle of the day and recovered at night. In 2 or 3 days the most severely affected plants wilted permanently and later became dry and brown. Occasionally, plants existed in a semiwilted condition for several days or weeks. Others did not become completely girdled and did not wilt. In damp portions of the greenhouse where the stems of the plants were wet, lesions were seen that extended up the main stem and onto the side branches.

## VARIETAL SUSCEPTIBILITY

Three varieties of snapdragons were being grown in the greenhouses, all of which were susceptible to the disease, but there was considerable variation in the varieties as to the degree of susceptibility. The variety Cheviot Maid was most resistant to the disease, only 20 per cent of the

plants being killed. Thirty per cent of the variety Jenny Schneider were wilting, and more than 75 per cent of the variety Roman Gold were destroyed by the disease. The 3 varieties were not grown in separate parts of the greenhouse but were mixed indiscriminately through the beds.

### ORGANISMS ASSOCIATED WITH THE DISEASE

Cultures were made from a number of diseased plants found in the grower's greenhouse and 3 organisms were consistently isolated. One was a bacterium. Another was a fungus, later identified as *Cephalosporium acremonium* Corda. The third organism, also a fungus, was a species of Phytophthora. These appeared repeatedly in the plates, either as pure cultures or as mixed cultures. Each organism was grown in pure culture and used in an inoculation study. The results of this study (Table 1) show conclusively that the species of Phytophthora was the organism causing the disease and the other two were secondary invaders.

TABLE 1.—*Infection results obtained by inoculating snapdragon plants with Phytophthora, Cephalosporium, and bacteria singly and in combination*

| Type of inoculum placed around stems of the plants | No. of plants inoculated | No. of plants diseased |
|---|---|---|
| Phytophthora on corn-meal-sand medium | 5 | 5 |
| Cephalosporium on corn-meal-sand medium | 5 | 0 |
| Bacterial spore suspension | 5 | 0 |
| Phytophthora and Cephalosporium each on corn-meal-sand medium | 5 | 5 |
| Phytophthora on corn-meal-sand medium and bacterial spore suspension | 5 | 5 |
| Cephalosporium on corn-meal-sand medium and bacterial spore suspension | 5 | 0 |
| Phytophthora and Cephalosporium each on corn-meal-sand medium and bacterial spore suspension | 5 | 5 |
| Check plants | 5 | 0 |
| Total | 40 | 20 |

In all cases where Phytophthora caused plants to wilt it was recovered in pure or mixed cultures. There seemed to be no perceptible degree of difference in pathogenicity where the Phytophthora was used in pure culture or in association with the other 2 organisms.

After it had been determined that the Phytophthora was the pathogenic organism, 2 additional groups of snapdragon plants were exposed to cultures of the fungus grown on corn-meal-sand medium. The inoculum was placed around the stem at the soil line and kept moist. Out of 55 plants

thus treated all were found to be diseased by the Phytophthora fungus. Fifteen check plants remained in a healthy condition. On the plants that were affected after exposure to the fungus, the symptoms of the disease were identical with those first observed in the greenhouse at San Leandro. Some of the plants began to wilt about 4 days after being exposed to the fungus growing on the corn-meal-sand medium. Other plants remained free of any wilting for as long as 2 weeks after being exposed. Lesions ranged from a light yellow or light grayish, water-soaked area to a dark brown, soft mass. On all of the plants the cortex was badly decayed and slipped off the woody xylem core of the stem very easily.

### DESCRIPTION OF THE CAUSAL FUNGUS

The mycelium is a long, very irregularly branched tube showing a few cross walls when old. On artificial media the mycelium presents a warty appearance. The hyphae are very granular and slightly yellow. Within the host tissue the mycelium is not so irregularly shaped as it is on artificial media. The hyphae range in diameter from 4 to 15 μ. On potato-dextrose agar the mycelium is dense, with an abundant aerial growth. On corn-meal agar the growth is very dense with much aerial growth. On oatmeal agar there is practically no aerial mycelium but there is a heavy growth on the surface and within the agar.

Conidia are fairly abundant on both potato-dextrose and corn-meal culture media. In size, they measure 20 to 25 by 30 to 45 μ. They are ovate in form, and each shows a distinct papillum. In color they are a light yellow, with a granular content. The conidia are terminal, being borne on slender hyphae branching off the larger mycelium.

Occasionally, the conidia have been observed germinating. Usually the germ tube arises near the apex of the conidium. A number of conidia have been observed producing swarm spores. The spores began moving within the sporangium and were extremely active for a half hour. Following this, a vesicle was seen to form near the papillum and the swarm spores swam into it. The vesicle was ruptured in a very short time and the spores swam free in large numbers.

Antheridia and oogonia were present both in cultural media and within the host tissues. The antheridium was roughly semispherical in form and lodged against the side of the spherical oogonium. The antheridium was not located at the base of the oogonium, and no germ tube was observed entering the oogonium. Oospores were numerous in the decayed tissue of the host plant. Apparently they form after the tissues are in an advanced stage of deterioration. In newly invaded tissues the mycelium can be seen in the cells. As the decay progresses the cells of the host turn brown and

completely collapse into a soft mass. The mycelium cannot be seen in this brown mass, but the oospores appear at this stage of decay.

### TAXONOMY OF THE CAUSAL ORGANISM

The morphology of the fungus, as described above, shows clearly that it is one of the Phytophthorae. After a study of the oogonia, oospores, and conidia was made, the fungus was closely compared with several well known species of the genus. *Phytophthora mexicana,* described by Hotson and Hartge (3), was found to differ from the snapdragon fungus in having longer and more narrow conidiophores. Also, *P. mexicana* is described as having the antheridia at the base of the oogonia, thus differing from the fungus described in this paper. *P. parasitica* var. *rhei,* described by Godfrey (2), produces haustoria, while no such structures were seen in the snapdragon tissues. The fungus on snapdragons was also found to differ from *P. syringae* in the size of conidiophores as described by Bruyn (1). Rosenbaum (4), in his studies of the genus Phytophthora, divides it into 3 groups, based on the position of the antheridium with reference to the oogonium. By this classification, the fungus found on the snapdragons belongs in the *cactorum* group. A comparison of the snapdragon fungus with various species of the *P. cactorum* group, as outlined by Rosenbaum, resulted in the conclusion that the Phytophthora isolated from the snapdragons is closely allied to if not identical with *Phytophthora cactorum* (Lebert & Cohn) Schröt. This conclusion is further strengthened by a study of a description of the species *P. cactorum* as given by Stevens and Plunkett (5).

### HISTOLOGICAL STUDIES OF DISEASED HOST TISSUE

Sections of diseased snapdragon stems were fixed, sectioned, and stained for study. An examination of stained slides showed that when the fungus had thoroughly invaded the stem the outer tissues had collapsed entirely. The epidermis was the first tissue to be invaded, following which the fungus penetrated rapidly through the cortex and phloem tissue to the cambium. Eventually the fungus made its way into the xylem, especially along the medullary rays. As the lesion enlarged around the spot of initial infection, the fungus traveled first in the cambium layer. Following the invasion of the cambium cells, the fungus penetrated the phloem and cortical cells. As the fungus progressed in its growth all of the cells except those in the xylem area collapsed and formed a brown shapeless mass. Among these collapsed cells were many oospores. (Fig. 1, B.)

### CONTROL MEASURES

In attempting to find a means of eliminating the trouble on the snapdragons, the greenhouse was examined for sources of infective material.

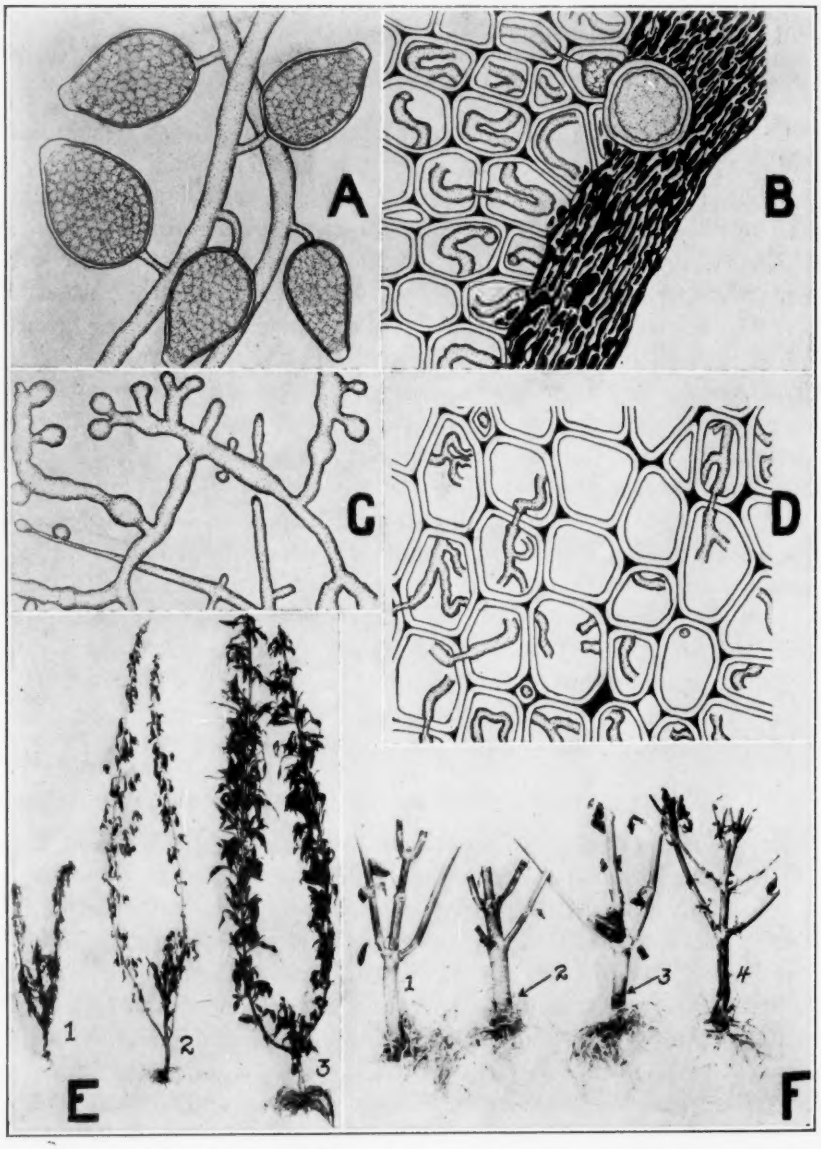


FIG. 1. A. Conidiophores of *Phytophthora cactorum* showing appearance of terminal papilla. B. Cross section of diseased snapdragon stem showing collapsed cortical tissue, an oogonium, and an antheridium. C. Mycelium grown on potato-dextrose agar culture. D. Cross section of xylem tissue showing mycelium within the cells. E. (1) Snapdragon plant stunted and killed by an attack of *P. cactorum;* (2) wilted plant attacked just before the blooming stage; (3) healthy plant. F. Roots and main stems of snapdragon plants: (1) healthy; (2) small lesion; (3) girdled by lesion; (4) cortical tissue sloughed off main stem, exposing woody xylem.

After a thorough search and tests of the soil and water used in the greenhouses, the parasite was located in the compost pile from which soil was taken to fill flats used at the time of transplanting the seedlings from the seed bed. Flats full of the compost soil were placed in a steam autoclave and subjected to a steam pressure of 10 pounds for half an hour. The soil was treated only once and was used for transplanting of plants from the seed bed as soon as it had cooled. This treatment completely killed the fungus, as shown by the fact that the disease did not affect plants grown in soil so treated and used in the transplanting flats. Soil believed to be free of the fungus was used to replace old soil in the large greenhouse beds. No disease appeared when plants from the steam treated flats were transplanted into the greenhouse.

## SUMMARY

(1) A hitherto unreported wilt of snapdragons is described as to symptoms.

(2) Three organisms, an undetermined bacterium, *Cephalosporium acremonium* Corda, and a Phytophthora species were isolated from diseased snapdragon tissues. Inoculation experiments using the 3 organisms showed that the Phytophthora species was the pathogenic organism causing the disease.

(3) The fungus is described as to its morphology. A comparison with known Phytophthora species shows that the organism here described is closely allied to if not identical with *Phytophthora cactorum* (Lebert & Cohn) Schröt.

(4) A histological study of the fungus in the plant tissue is discussed.

(5) Control measures which have totally eliminated the disease from the affected greenhouses are given.

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# A METHOD OF RECORDING THE DISTRIBUTION OF COPPER DUSTS OR SPRAYS ON LEAVES

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(Accepted for publication June 14, 1933)

When experiments were undertaken recently to determine more precisely some of the factors that increase the efficiency of potato spraying, it was evident at once that some more direct method than field tests was desirable with which to make preliminary studies. Questions such as these were being raised:—What pressure is most profitable? How many nozzles to the row are desirable? What kind of a spray boom is most efficient? How should it be adjusted? What kind of nozzles should be used? How much material should be applied? Similar questions are being asked in connection with dusting. If it were possible to spray or dust small areas of a field with each of several arrangements of nozzles and booms and then determine at once the kind of distribution obtained, progress in testing modifications of the spraying and dusting apparatus would be facilitated.

Working with this aim in view, there was developed a method of making prints on paper directly from the sprayed leaves to show the distribution of the copper. As a number of other workers have shown an interest in this method, it is now described in the hope that it may prove useful in similar cases.

The paper is prepared by dipping it in an acidifying solution of potassium ferrocyanide in water. The acid is added to render the copper of Bordeaux mixture soluble. The potassium ferrocyanide in the paper reacts to give the familiar brown precipitate (cupric ferrocyanide, Hatchett's brown) well-known to those who have used this reagent for testing Bordeaux mixtures for soluble copper. This precipitate remains adherent to the surface of the paper, where it produces a brown spot the size and shape of the Bordeaux spot on the leaf.

The concentration of the solution used apparently is not very critical. Two grams of potassium ferrocyanide and 5 cc. of acetic acid to 100 cc. of water were satisfactory. Nitric acid (2 cc.) may be used in place of the acetic acid.

Iron reacts with potassium ferrocyanide to give Prussian blue, so that this paper may also be used in tests for iron. For this reason iron must be avoided both in the paper and in the utensils used in the preparation of it. The vessels used to hold the solution should be glass or glazed earthenware. While extensive tests were not made, there were numerous disappointments at the beginning, apparently due to the presence of a small amount of iron

in the paper, especially in mimeograph paper. When tests were made later with a series of samples of different kinds of paper, bond papers were selected as most satisfactory.

After the paper has been thoroughly wetted with the solution, it may be kept moist until ready for use or, if dried, must be moistened again before it is used. It may be readily carried about and kept moist in paraffined envelopes, but here, again, metal fasteners must be avoided.

Samples of potato leaves may be collected to show the distribution of spray or dust on different parts of plants or on any particular part, as desired, and should be allowed to lose their turgescence before they are in best condition for printing. The moist paper is then placed on a cotton pad covered with muslin. The leaves or leaflets are arranged on the paper as desired with the upper side down. A second sheet of the prepared paper is placed on top of the leaves if a print of both sides of the leaves is to be made. Another cotton pad is then added. There are then in order a cotton pad, a prepared paper, a layer of leaves, another prepared paper and another cotton pad.

Immediately after this is done the whole pile is placed in a press. An old letter press is convenient for the purpose. Medium pressure at the beginning seems to avoid running together of the spots if the paper is too wet. Free drops of water on the paper are to be avoided for the same reason. Five to 10 minutes in the press is sufficient. The press should be tightened occasionally during this time.

After pressing, the paper is taken out and washed in water to remove the excess chemicals and thus avoid further reaction. It may then be dried to form a permanent record. The brown spots on the paper represent the Bordeaux spots on the leaf. One sheet records the spots on the upper side of the leaf, the other those on the lower side.

Any iron present, such as impurity in the copper sulphate, is recorded in blue. Incidentally, labels written on this paper, before washing, with ordinary fountain pen ink are changed to a permanent blue, which does not wash out.

Photographs of prints made from sprayed leaflets are shown in figure 1. Only a sample can be shown that was selected from a large number to represent the results obtained in comparing different pressures. The 3 prints of the upper row represent the upper side of the leaflets and those in the lower row the under side of the same leaflets. The first print at the left represents a sample taken from leaves sprayed at 400 pounds' pressure, the second, samples sprayed at 200 pounds' pressure but with nozzles with a larger orifice to apply as much material as at 400 pounds' pressure and the third shows a sample at 200 pounds' pressure with the same nozzle

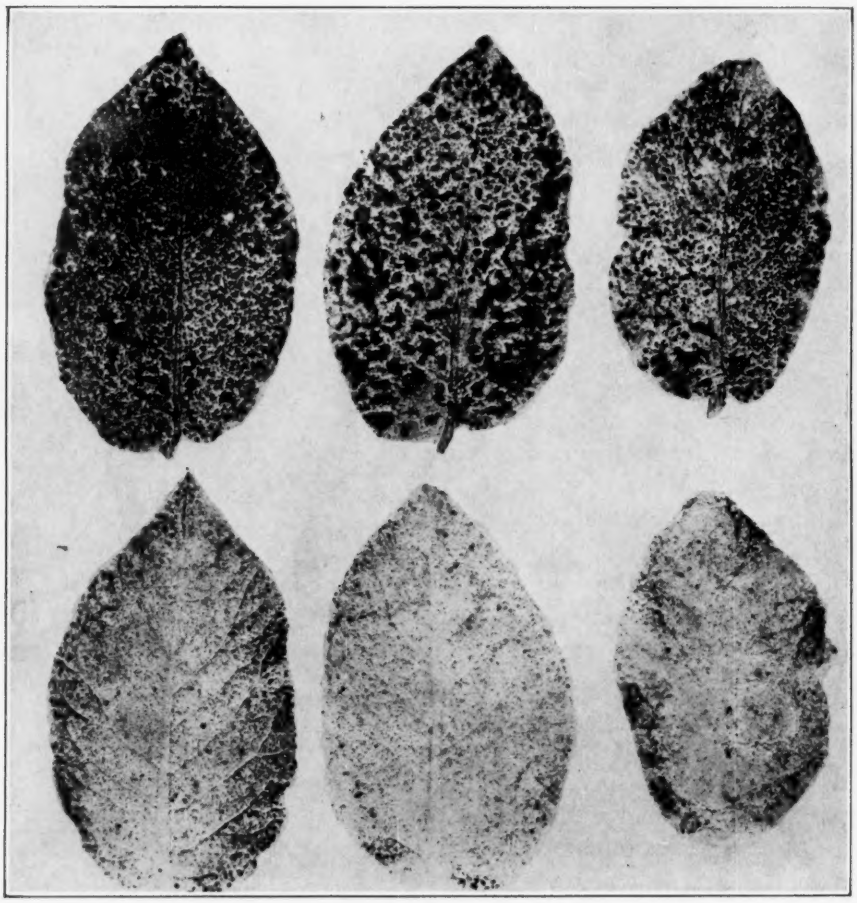

FIG. 1. Prints of leaflets sprayed with Bordeaux. Upper sides of the leaflets are shown in upper row, and under sides of the same leaflets in lower one. Beginning at the left, the first leaflet was sprayed at 400 pounds' pressure, the second at 200 pounds' pressure with the same amount of material as the first, and the third at 200 pounds' pressure with a smaller amount of material but with the same size nozzle orifice as the first.

orifice as at 400 pounds. The lower row shows the under sides of the leaflets in the same order.

It can be seen that the drops of Bordeaux have run together more when the larger amounts are applied at the lower pressure.

Some difficulty was encountered late in the season in trying to get good prints. This was due to the accumulations of copper on the leaves to such an extent that they were completely covered. To get good prints of any one spraying, it is, therefore, desirable to apply the tests to plants that have not previously been sprayed.

To test how accurately prints made in this way represented the original spots as to size and shape, 100 measurements of the diameters of original spots were compared with measurements of the prints of the same spots. The spots after printing averaged 5.6μ ± 2.1 larger, indicating that the difference is small in any case and probably mostly due to errors in measurement. This was done with prepared paper that was just moist. With an excess of moisture present some enlargement or running of the spots occurs.

It would also be desirable to represent the relative coverage secured by different methods of spraying in some quantitative way. While one can make a fair estimate by comparing the appearance of prints made of leaves sprayed in different ways, such differences would be difficult to describe adequately without some way of measuring them. A few measurements have been made to test the practicability of the method. The spray spots on the prints were too small to measure conveniently directly. They were, therefore, enlarged. Outline drawings were made, using a camera lucida with a low power lens. To get the percentage of the total area covered by spray, the spots were cut out and weighed. A planimeter could be used for the same purpose.

Such measurements were made from a series of prints made in 1929 in spraying experiments. There were 3 series of plots: No. 1 sprayed at 200 pounds' pressure, No. 2 at 400 and No. 3 at 200 pounds, but with nozzles

TABLE 1.—*Showing the gain in yield and in percentage of leaf area covered by spraying at higher pressures and with larger nozzle openings*

| Series compared | Pressures and gallons per acre | Average gain in percentage of leaf area covered | Average gain in yield. bushels per acre |
|---|---|---|---|
| No. 2 over No. 1 | 400 pounds (128 gals.) over 200 pounds (77 gals.) | 38.3 ± 1.1 | 36.5 |
| No. 3 over No. 1 | 200 pounds (128 gals.) over 200 pounds (77 gals.) | 18.4 ± 1.0 | 18.1 |

of larger orifices, so as to apply as much material as at 400 pounds' pressure with the smaller-nozzle orifice. The results of this series of measurements are given in table 1, with differences in yields for comparison. The area figures in the table are the average differences for 10 measurements from the prints of the upper surfaces of the leaves at each pressure. This probably is an inadequate number, considering the variability likely to be encountered, but will at least indicate the possibilities of the method.

Thus it appears that the method devised of making prints from leaves, using paper prepared by moistening with an acidified potassium ferrocyanide solution, offers considerable promise. Such prints may be kept as a permanent record, and may serve as visual illustrations or may be used as a basis for more accurate study of the comparative covering secured with different apparatus or methods or on different parts of the plant.

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## PHYTOPATHOLOGICAL NOTES

*Physiologic Forms of Puccinia graminis tritici in Kwangtung, Southern China.*[1]—The black stem rust of wheat (*Puccinia graminis tritici* E. et H.) is the most destructive disease of wheat in Kwangtung Province, Southern China,[2] and is becoming the limiting factor in profitable wheat growing. From observations thus far made, there are no susceptible species of barberry of any importance in this general region. Control by the eradication of the alternate host, therefore, is out of the question. Although dusting with sulphur has been demonstrated experimentally to control stem rust in other countries,[3,4,5] it is not feasible as a practical control measure in China. Apparently the only possible practical control measure will be by growing resistant varieties.[6,7] Consequently, a study of physiologic specialization of the pathogen is important.

During March and April, 1932, wheat infected with the uredial stage of rust was collected in various localities in Kwangtung Province and used for making inoculations on the 12 differential hosts listed by Stakman and Levine.[8] The usual methods were followed in inoculating, incubating, etc. The results are summarized in table 1.

It will be seen that there are 6 physiologic forms. Accession numbers 1 and 2 correspond with forms 15 and 9, respectively, as described by Stakman and Levine.[8] The remaining 4 have never before been described and appear, therefore, to be entirely new. In the latest key issued by Stak-

[1] The author is greatly indebted to Dr. E. C. Stakman and Dr. M. N. Levine, of the U. S. Department of Agriculture and the University of Minnesota, U. S. A., for furnishing seed of the differential wheat varieties and comparing the rust reactions with those recorded in their keys.

[2] Tu, Chih. Notes on diseases of economic plants in South China. Lingnan Sci. Jour. **11**: 489–504. 1932.

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TABLE 1.—*Reaction of differential hosts of wheat to physiologic forms of stem rust in Kwangtung, Southern China*

| Acc. No. | Place of collection | Infection type | | | | | | | | | | | | Physiologic form |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Little Club | Marquis | Kanred | Kota | Arnautka | Mindum | Spelmar | Kubanka | Acme | Einkorn | Vernal | Khapli | |
| 1 | Lingnan Farm, Canton | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 1 | 15 |
| 2 | Shih Lung | 4 | 4 | 0 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 1 | 9 |
| 3 | Tseng Cheng | 4 | 4 | 0 | 2 | 1 | 4 | 4 | 1 | 4 | 1 | 1 | 1 | 132 |
| 4 | Do. | 4 | 4 | 0 | 0 | 1 | 2 | 2 | 4 | 4 | 0 | 1 | 2 | 133 |
| 5 | Shiu Kwan | 4 | 4 | 0 | 0; | 0; | 0; | 0; | 1 | 3 | 3 | 3 | 1 | 134 |
| 6 | Do. | 4 | 4 | 4 | 0; | 0; | 3 | 2 | 4 | 3 | 1 | 1 | 1 | 135 |

man and Levine (mimeographed) they would be designated as forms 132, 133, 134, and 135. It seems noteworthy that 2 of the 6 forms appear to be identical with forms occurring in North America, while 4 of them are different. It would be interesting to know whether the latter, forms 132 to 135 inclusive, exist in other regions of the world also, or whether they are peculiar to Southern China. Further studies on the number, distribution, and origin of forms in various regions of China are very desirable, both from a practical and a scientific point of view.—CHIH TU, Honan University, Kaifeng, Honan, China.

*Mealy-Bug Wilt and Green Spot in Jamaica and Central America.*[1]—The situation with respect to these two diseases of pineapple was observed by the writer in Jamaica, Guatemala, and Spanish Honduras in the fall of 1932. In Jamaica the situation was complicated by the absence of Smooth Cayenne, the variety of pineapple on which previous studies have been made, and the plants seen were not growing in as high a state of cultivation as is the case in Hawaii.[2,3]

[1] Published with the approval of the Director as Technical Paper No. 70 of the Experiment Station of the Pineapple Producers Cooperative Association, University of Hawaii.

[2] Carter, Walter. The pineapple mealy bug, *Pseudococcus brevipes*, and wilt of pineapples. Phytopath. 23: 207–242. 1933.

[3] Carter, Walter. The spotting of pineapple leaves caused by *Pseudococcus brevipes*, the pineapple mealy bug. Phytopath. 23: 243–259. 1933.

The absence of Smooth Cayenne is in itself significant, however, since the prevailing opinion among informed persons in Jamaica is that the variety disappeared on account of its extreme susceptibility to "wilt," the term being understood only in a generic sense. The variety Red Ripley, though highly regarded in Jamaica, is likewise much more rare than it was at one time. The variety most commonly grown is variously known as Cheese, or Sugar Pine. A number of other varieties are present in very small numbers in private gardens.

The conclusion was reached that mealy-bug wilt is present in Jamaica and that Red Ripley is becoming rare on that account. It is altogether likely that Smooth Cayenne has disappeared from cultivation there for the same reason. Cheese Pine is unquestionably much more resistant to mealy-bug wilt than any other variety seen. Even with high populations of mealy bugs this variety is vigorous and apparently wilt resistant.

Green spotting was encountered in Jamaica on several varieties. On those with pure green leaves the spots were typical of those described from Hawaii; on those whose leaves contained red and brown color, the color of the spots was modified. Data on green spotting are summarized in table 1.

TABLE 1.—*Data on green spot of pineapples in Jamaica, September, 1932*

| Location | Variety | Green spots | Type of spot |
|---|---|---|---|
| Manor House | Cheese | + | Typical |
| | Porto Rican | + | Typical |
| | San Clarke | + | Flat; brown when old |
| | Cowboy | + | Spots show as darker brown areas on leaves that are deep green with brown underlay |
| Buff Bay | Cheese | − | |
| Orange Bay | Cheese | − | |
| Hope Gardens | Cheese | − | |
| | Ripley | − | |
| Half Way Tree | Cheese | + | Typical |

In Guatemala, where collecting was done under a wide variety of environment, no green spotting was encountered, even though on the lowlands the pineapple mealy bug (*Pseudococcus brevipes* Ckl.) was very common and on a veriety that in Jamaica showed green spotting, *i.e.*, Cheese Pine. Since, in the areas where the mealy bug was common, the variety grown was almost exclusively Cheese Pine, the absence of mealy-bug wilt was not surprising in view of the Jamaican experience.

In Spanish Honduras, green spotting was found only in one place, The

United Fruit Company garden near Progresso. There the mealy bug was rare, but green spots were found on 3 varieties. It is perhaps significant that transfer of planting material from Hawaii to this area had taken place when The United Fruit Company made the variety collection. As a matter of fact, areas where green spotting has been observed have all shared in the transfer of planting material at one time or another. Since mealy bugs are readily transferred in this manner, the distribution is readily understood. A considerable amount of Smooth Cayenne material was transferred from Jamaica to Hawaii some years ago. There is some evidence that transfers had been made from Florida to Jamaica even earlier. Some interesting speculation as to the origin of mealy-bug wilt and green spotting is possible when its present distribution is considered—whether the two diseases have been transferred from one of these places to the others through the medium of mealy-bug-infested planting material, or whether, since the capacity to produce both appears to be inherent in the mealy bug, the greater severity and incidence of these diseases in Hawaii are associated merely with the higher state of pineapple cultivation existing there.

No typical wilt was observed in Spanish Honduras, though, again, the variety was principally Cheese Pine. In The United Fruit Company garden, mealy bugs were extremely rare, as was the case in the same company's experiment-station garden at Lancitilla, where a small pure stand of Smooth Cayenne was growing without any evidence of wilt whatsoever.—WALTER CARTER, Pineapple Experiment Station, University of Hawaii, Honolulu, Hawaii.

## BOOK REVEIWS

Paul Sorauer, *Handbuch der Pflanzenkrankheiten.* Herausgegeben von Dr. O. Appel. Volume I. Die Nichtparasitären und Virus-Krankheiten. Part I. (Nonparasitic diseases.) Pp. X × 592. Paul Parey, Berlin, 1933. 46.00 Gold Marks.

Sorauer published his manual on plant diseases a little more than half a century ago. All the facts were then crowded into a relatively small book, while the present edition consists of 6 formidable volumes, of which the first is issued in two parts of approximately 500 pages each. The subject matter of the first volume closely resembles Sorauer's first manual as it deals with non-parasitic and virus diseases. Through the most able editorship of Dr. Appel and his contributors there has been made available to the reader a wealth of information which makes the present edition an indispensable item in the library of the pathologist.

Part I of the first volume deals with nonparasitic diseases. It is divided into two sections. The first section is introductory and general in scope, dealing with the history of plant pathology, the concept of disease and the many-sided relationships between disease factors and environment. It is by far the most fascinating part of the first volume as it furnishes the physiological and ecological background for plant-disease study. It is written in a clear and forceful language, much easier to read than "Sorauer of old" whose long sentences often remained a mystery to those who boasted of only a high-school or college acquaintance with the German language. The second section of the book takes up: plant nutrition in relation to disease, climatic factors as causes of nonparasitic diseases and temperature extremes (heat and cold) as factors in death and disease.

The history of plant pathology is traced back to 4000 B. C. and the treatment of the subject is as complete as 79 pages of crowded print can make it. Its wealth of information far exceeds that found in related books and the story makes interesting reading not only to the pathologist but to all those interested in the progress of biological science.

The subject of general pathology, pp. 80–198 in the book, is intended as an introduction to the study of specific diseases. Following a brief, though clear, discussion on the concept of disease, approximately 20 pages are devoted to disease symptoms. The following 7 major types are listed and illustrated:

1. Wilting.
2. Discolorations (general and partial discoloration; spotting).

3. Death phenomena (dropping and withering of plant organs, rots, and decays).

4. Form changes (changes in size, simple form changes, atrophy, proliferation, etiolation, monstrosities, new formations).

5. Wounds (wounds due to atmospheric influences, gnarls and cancers, injuries resulting from animal feeding).

6. Deposits and excretions (honey dew; root pressure exudate, gum and resin flux, mucilage formation).

7. The presence of epiphytes and parasites as principal symptoms.

Two principal disease causes are recognized: a. inanimate nature acting either directly or indirectly, and b. living organisms, especially fungi. As principal inanimate causes the authors list unfavorable climatic and soil factors, presence of wounds and chemical influences.

The subject of parasitism and pathogenicity is treated in great detail. In the chapter on disease classification the different principal systems, especially those of Appel and Westerdijk and Wetzel are described and evaluated. Since a satisfactory explanation for the cause of disease is only obtained if the behavior of host and parasite to their environment is recognized and correctly evaluated, the part of the book dealing with ecological factors in relation to disease is most interesting. The authors give first a brief discussion on general plant ecology treating in some detail the subjects of: phenology, constitution and disposition, predisposition, degeneration and running out, immunity and resistance. In the chapter "on pathogen and environment" the subject of aggressiveness and virulence is taken up with special reference to the effect of temperature and nutrition. Attention is being called to the oligodynamic fertilizers such as manganese, boron, etc., which, in small quantities are stimulating, while larger applications are poisonous. Deficiency in boron may cause dry rot in beets, but, on the other hand, reduce Helminthosporium leaf spot of barley.

About 20 pages of the book are devoted to a discussion of the relation of animal parasites to environment; the mass appearance of insects and the subsidence of epidemics.

A diagnosis of nonparasitic diseases is difficult at best and often it is quite impossible if such material is sent to the laboratory away from its environment. To aid in the identification of such diseases pathologists have, in the course of years, accumulated a wealth of useful data and information which has been compiled and made available in the chapter on "plant nutrition and plant diseases." The authors treat first general symptoms, pointing out the fallacies in diagnosis from color symptoms. Under biochemical symptoms we become acquainted with pathological mineral metabolism; pathology of under-nutrition and other deficiencies, pathology of over-nutrition; pathological stimulation, pathological enzymatic processes and auto-

intoxication. Approximately 50 pages are devoted to mineral deficiency and the resulting effects on the plant. The need for a well-balanced soil solution has always been recognized, but it is only after reading this chapter in the book that one begins to see the numerous pitfalls the pathologist encounters in trying to diagnose a sick field or a sick individual growing on a deficient soil. A certain number of plants demand a finer ecological balance in relation to their environment than others. Notable examples are those that cannot grow on lime or acid soils.

With a knowledge of plant nutrition in relation to plant diseases for a background, the authors discuss in some detail a few of the economically important nonparasitic diseases: heart and dry rot of beets, dry-spot disease of oats, white head of oats and grasses, and reclamation (or moor) disease.

About one fourth of the book is given to a discussion of climate and weather as causes of nonparsitic diseases. Dr. Hiltner discusses first the subject of light and radiation, treating in some detail photoperiodism, time of seeding, light deficiencies, the effect of varying $CO_2$ content of the air, and cosmic influences. Next he takes up temperature relations giving numerous examples of the effect of temperature excess or deficiency on the development of economic plants. Often the effect of temperature deficiencies is indirect. Thus, the flower abortion in grapes during a cold spring is not the direct effect of the cold but is due to a loss of balance in the absorption of minerals and photosynthesis. Of more than passing interest is also the chapter on water movement. Abnormally high humidity of the air is responsible for the formation of intumescences, abnormal cork proliferation, and warty protuberances, on stems. On the other hand, a very dry air may cause injuries to leaves and buds and is in part responsible for dry spot diseases, withertip and Weissährigkeit (white heads in cereals). The last 100 pages of the book deal with temperature extremes as cause of death and disease in plants. The various theories on ice formation in plants are critically discussed and evaluated. What changes take place in the cell as the temperature drops, and is the plant able to endure such changes? As positive changes are listed: intercellular and intracellular ice formation, dehydration of cell colloids and changes in the sale concentration of the cell. Often the manner of defrosting exposed plants determines whether they will live or die. An explanation for this curious phenomenon is as yet lacking. Another unsolved problem is the freezing of plants above actual freezing temperature as frequently happens with tropical plants. Freezing often results in injury to both herbaceous and woody stems, causing splitting and malformations. Seeds may suffer decrease in germinability and frozen or injured organs often form favorable places for parasites to attack.

Injury and even death to plants and plant organs may be occasioned by temperatures above the normal. A common heat injury is the burning of

foliage often observed in greenhouses after a period of intense solar radiation. Leaf drop due to heat sometimes occurs in the spring and may be so severe as to produce a pathological aspect. The actual cause of this phenomenon is still obscure. Excessive cold and heat alike tend to weaken the plants and make them more easy prey to attack by parasites.

As may be seen from the above discussion the first part of Volume I deals to a large extent with normal plant physiology, an understanding of which is essential in the correct diagnosis of nonparasitic plant diseases. The literature citations are numerous, though mostly German. The illustrations are pertinent and mostly from the pen of Sorauer, thus vouching for their excellence.—ERNST ARTSCHWAGER.

*Recent Advances in Agricultural Plant Breeding.* By H. Hunter and H. Martin Leake. P. Blakiston's Son & Co., Inc., Philadelphia, 1933. 361 pp. $4.00.

This volume, with a foreword by Sir Rowland Biffen of Cambridge, is written by men with long and fruitful experience in plant breeding and agricultural science. Hunter was in charge of plant breeding (barley) in Ireland for some 18 years and now has charge of oats breeding in the Plant Breeding Institute at Cambridge. Leake has intimate, first-hand knowledge of tropical crops, having served as Director of Agriculture, United Provinces, India, and as Principal of the Imperial College of Tropical Agriculture, Trinidad.

In his foreword Professor Biffen refers to the attempts now being made, by those engaged in economic plant breeding, to mitigate the losses caused by fungoid and insect pests. He states that "a considerable body of evidence has now been accumulated, that, even, if it is not often known what susceptibility and immunity to attack are actually due to, these factors are nevertheless heritable. Thus in section after section of this book it will be found that breeding for disease resistance is part and parcel of the investigator's work. This is notably the case with such crops as cotton and wheat. It is now clear that only continuous effort along clearly defined lines is required for obtaining a far greater measure of control over plant diseases than was ever dreamed of before Mendel's work set the course for the modern plant breeder."

The authors have adopted an interesting and significant basis for the selection of material and have "attempted to present in a connected form the results of only such investigations as have advanced beyond the limits of purely academic interest and the outcome of which has been the introduction of improved varieties into general agricultural use."

The 17 chapters of the book are grouped in two parts, (1) crops of the temperate regions, and (2) crops of the sub-tropical and tropical regions.

In the first 8 chapters, plant-breeding results with wheat, barley, oats, flax, the potato, forage grasses, red clover, and white clover, and with root crops —mangel, swedes and turnips—are described. Chapters 9 to 17 deal with beverages—coffee, cacao, tea; sugar cane, fruits, including citrus and allied genera and the banana; tobacco, the opium poppy, the tropical cereals: maize, rice, and sorghum; rubber, fibres: cotton, sisal, New Zealand flax, jute; and oil plants: coconut, oil palm, castor, groundnut, sesame, and the soy-bean. It may seem a bit strange to plant pathologists and plant breeders in the northern United States to see maize classed as a tropical cereal, but this is perhaps not illogical from the standpoint of workers in the British Empire, in whose hands this book will probably find its greatest usefulness. The literature of plant breeding with tropical crops has heretofore been widely scattered and not readily available, and the authors have rendered a real service in bringing together much of this very interesting material.

Throughout the book emphasis is given to the problems, methods, and results in breeding crop plants for resistance to plant diseases and insect pests. Plant pathologists, plant breeders, entomologists, and agronomists, who are interested in this field will find the book a very useful and convenient reference work.

In the chapters on wheat, considerable space is devoted to discussion of the early studies of Biffen on rust resistance and to more recent work of Stakman, Hayes, Waldron, Clark, McFadden, Goulden, *et al.* Reference is made also to the studies of Painter and his associates at the Kansas station, on the resistance of varieties of winter wheat to Hessian fly.

The work of Danish, Irish, and English investigators is featured in the chapter on barley, which includes a succinct discussion of malting quality.

Studies of resistance to the smuts, rusts, grey-spot disease, and to the fruit fly, *Oscinella Frit*, Linn., are described in the chapter on oats, where the publications of Reed, Gaines, Stanton, Murphy, and other American workers, and of European investigators at Cambridge, Oxford, Aberystwyth, and Svalof are cited.

Studies of resistance to wilt and rust are described in the chapter on flax, in which some of the papers of Bolley, Stakman, and other American workers, and of Pethybridge in Ireland, are reviewed.

Studies of blight resistance by Müller, Reddick, *et al.*, and of wart disease by Salaman and Lesley, and virus diseases by K. M. Smith, are described in the chapter on the potato.

A strain of rye grass known as Hawkes Bay is described that shows marked resistance to leaf rust, *Puccinia coronata.* Davies *et al.* in Wales have described varieties of swedes resistant to the finger and toe disease, *Plasmodiophora brassicae.*

Many additional cases of resistance to plant diseases and insect pests of crops of the sub-tropical and tropical regions are described in Part II. Space permits mention of only a few examples. Thus, Java strains of cacao exhibit varying, but marked, degrees of resistance to disease, particularly to *Acrocercops cramerella,* the cacao moth, and to *Helopeltis.*

The stimulus that led to the very extensive work with sugar-cane seedlings arose from the disease susceptibility of the older canes. Resistance to the sereh disease in Java has been obtained by using the resistant *Saccharum spontaneum* as one parent in crosses. The Indian canes generally exhibit a marked degree of resistance to mosaic and, for this reason, Uba has become the predominant cane in South Africa. There are notable differences in the degree of resistance of citrus fruits to gummosis and withertip.

Studies of resistance to black root rot, and to black shank of tobacco, are described. In India Leake observed a considerable range of susceptibility to the leaf fungus, Phytophthora, in different races of the opium poppy. The races from Central India are notably susceptible.

With reference to maize, the authors state that "the question of disease is of importance second only to the question of inherent capacity to yield." Studies of resistance to corn smut, rust, pink rot, and Stewart's disease, are described. Roubaud reports complete immunity from the corn borer for Dent de Cheval and partial immunity for Hatif d' Auxonne maize, in France. Marston, of the Michigan experiment station, is using the borer-resistant maize Amargo from South America, to produce resistant varieties adapted to Michigan conditions. The studies of Collins and Kempton, of the United States Department of Agriculture, on infestation of corn earworm as related to the length and thickness of husk, are briefly described.

Varieties of rice show different degrees of resistance to blast, *Piricularia oryzae,* though none are immune. Studies of Reed and of Tisdale, *et al.,* on resistance to kernel smut in sorghum, are given brief mention.

The cotton plant is, in all countries, subject to numerous diseases that, in more than one instance, have become the critical factor in economic cultivation. Among the varieties resistant to wilt are Dillon, Dixie, and Dix-Afifi. Strains of Pima and Acala cottons have been raised that are relatively resistant to cotton root rot. Harland has shown that Sea Island cotton responded to selection for resistance to *Bacterium malvacearum* E.F.Sm. Distinct progress has been made by cotton breeders in producing varieties resistant to insects. Thus, Harland has reported distinct differences in palatability of different species and varieties of cotton to the boll worms. Early maturing strains of long-staple cotton such as Express and Webber have helped make it possible to grow Upland varieties in the United States under boll-weevil conditions. In the West Indies, the Upland

group of cottons are all severely attacked by the blister mite. The Bourbon group is immune, while the Peruvian group shows varying degrees of susceptibility. In South Africa the attacks of the cotton jassid are so severe that it seemed probable that cotton cultivation throughout the southern portion of that continent was doomed. Worral and Parnell have selected resistant strains. One of these, U4, is markedly superior to all the others and in 1929–30 practically the whole of the low veldt was planted with it. Cambodia cotton, a type introduced into South Africa from India, has proved even more resistant to jassid than U4. A close correlation has been noted between resistance to the jassid and hairiness of the cotton plant. Certain strains of Sea Island cotton show a marked degree of resistance to leaf curl, a virosis.

There are noteworthy differences in resistance to yellow leaf, a virosis of New Zealand flax.

If any plant pathologist or entomologist doubts the importance of plant breeding as a control measure and if any plant breeder is unaware of the accomplishments and opportunities in breeding for resistance to diseases and insect pests, let him turn to "Recent Advances in Agricultural Plant Breeding" by Hunter and Leake, for inspiration and enlightenment.—JOHN H. PARKER, State College of Agriculture, Manhattan, Kansas.

*Environmental Factors in Relation to Plant Disease and Injury: A Bibliography.* By J. D. Wilson. Ohio Agr. Exp. Sta. Tech. Bul. 9. 203 pp. 1932. Price, $1.00.

In the progress of any branch of science a periodic inventory of the details amassed by many workers in diverse fields is essential in order that the facts may be consolidated and the true objectives more clearly perceived. Biologists generally have adhered far less closely to a *grand plan* of research than workers in the more exact sciences—physicists for example in their definite quest to understand the organization of matter. We say that the great aim of biology is "to explain life," but in plant pathology at any rate, we continue to regard as our serious business the descriptive and therapeutic phases of plant diseases. Perhaps this is because plant pathology is superficially only one of the phases of crop production and utilization; possibly another factor is the, as yet slight, progress of its synthetic development. To the extent that comprehensive inventories of accumulated data are prerequisite for the synthesis of principles, noteworthy aid to such synthesis in plant pathology is afforded by the publication of an extensive bibliography on the relation of environmental factors to plant disease and injury.

The subject of environmental relations to plant disease might appear as fairly extensive to even a well-informed layman. To a professional plant

pathologist it might well seem as too vast for any one to attempt except a student in Plant Path. I. For all the reviewer knows, the Wilson bibliography may be the outgrowth of one of these dreams such as ambitious beginners have. However, its present magnitude is convincing evidence that the author did not as predoctorate students often do, begin to "specialize" (*i.e.*, curb his ambition) as soon as the bibliography attained the burdensome size of several hundred entries. It requires rampant ambition to carry through to completion a bibliography of over 3,600 titles, as Wilson's does, and it takes an exceptional degree of organizing skill to provide effective clues to so great a body of data.

The 3,689 literature citations are arranged alphabetically by authors, and are numbered serially. Next follows an "environmental factor index," with the major factors listed under 24 headings including the usual meteorological and nutritional factors, and also categories not ordinarily included in the environmental complex—host characters, various deleterious agents such as toxins, smoke, dust, injuries from control operations, and the time factor in viability of pathogens. The major factors are subdivided into more specific categories, which are repeated in a second list with the serial numbers of the literature citations arranged under each topic. For example the major factor "Hydrogen-ion relations" has the following subdivisions: A. General; B. Regulating disease. 1. Acid soil favoring, 2. Alkaline soil favoring, 3. Cardinal soil reactions for disease development; C. Regulating growth. 1. Cardinal reactions of medium for fungi, 2. Cardinal reaction of medium for other plants, 3. Plant growth and distribution. The group C–1 contains 158 numbers each referring to a title in the literature under which cardinal reactions of media for growth of fungi are discussed. The relations of temperature to spore germination include some 200 citations, to growth nearly 500, lethal effects 200, etc.

A second index lists host plants alphabetically (by common and Latin names) and under each the diseases (descriptive name and Latin name of pathogen) and other injuries reported in literature. Under each disease or injury, the serial numbers of pertinent literature citations are given. Aside from the clues to literature that it furnishes, this index is a useful compilation of host plants and their diseases. As this index covers some 27 pages, a final host index listing the page on which each host appears is provided.

Noteworthily complete and skillfully organized, this bibliography will prove an indispensable reference work to student and professional plant pathologists.—FREEMAN WEISS, United States Department of Agriculture, Washington, D. C.

## FIRST INTERNATIONAL CONGRESS OF ELECTRO–RADIO–BIOLOGY

For the purpose of instituting among physicists, chemists, biologists, naturalists and doctors a close and profitable collaboration, indispensable for the advance of radio-blology considered not as a branch of radiology or of biology, but as a separate science in itself, the International Society of Radio-Biology is now preparing the organization of the First International Congress of Electro-Radio-Biology, which is to take place in Venice, in the Ducal Palace, in September, 1934, under the presidency of H. E. Count Volpi di Misurata, Minister of State.

All the subjects concerning oscillatory and corpuscular phenomena in relation to biology will be examined and discussed: ultra-sounds, electric waves, infra-red, light, ultra-violet, radium, penetrating radiation in its probable influences on the various manifestations of organic and organized matter and in applied laboratory work; photodynamic actions, long-distance actions of metals, Gurwitsch rays, phenomena of luminescence, radiations of radio-active salts in organic combinations; electric states of the atmosphere; spectrographie; influences of the radiating energy on heredity, etc. These are the subjects of the greatest importance, which will constitute a vast experimental and theoretic whole, to be treated by specialists in physics, in physical chemistry, in radiology, in biology, in botany, in zoology, in medicine, etc.

It is therefore a fundamental congress based on new and universal foundations, in which, for the first time, those whose studies are directed on all matters having a direct or indirect relation with the subject will exchange their own ideas, intending thus to reach a program of unification in the promising field of radio-biology.

The most prominent figures in physics, chemistry, and present day biology will take part, and they will give lectures on physics, chemistry and biology. After these lectures will follow talks of a more strictly radio-biological nature. Every radio-biological talk will be delivered separately by specialized physicists and biologists, through which the congress may, finally (after due consideration of the talks, discussions, and communications of the sections), be able to reach a radio-biological synthesis founded on severe principles of physics, physical chemistry, and biology.

The following will be present at the Congress and will give lectures and speeches: Emil Abderhalden (Halle A. S.); Belak (Budapest); Brunetti Rita (Cagliari); Arthur Compton (Chicago); Coolidge (Schenectady, N. Y.); Duceeschi (Padova); Glasser, Otto (Cleveland, Ohio); Gola

(Padova); Gurwitsch (Leningrad); Haskins (Schenectady, N. Y.); Magrou (Paris); Marinesco (Bucharest); Nadson (Leningrad); Nemenow (Leningrad); Palmieri (Bologna); Perussia (Milano); Pincussen (Berlin); Pugno-Vanoni (Padova); Rahn (Ithaca, N. Y.); Raman (Bangalore-India); Reche (Leipzig); Roffo (Buenos Aires); Rossi, Bruno (Padova); Swend Lomholt (Kopenhagen); Stempell (Münster); R. W. Wood (Baltimore), and other names will be added in successive communications.

In order to coordinate the tendencies of the congress-members in the best way possible, and at the same time to make present to the National Research Council the founding and the development of the initiative, those who desire more detailed information are invited to apply to the temporary head-office of the International Society of Radio-Biology, addressing their correspondence to: Dr. Giocondo Protti, Venice (Italy), Canal Grande-S. Gregorio 173.

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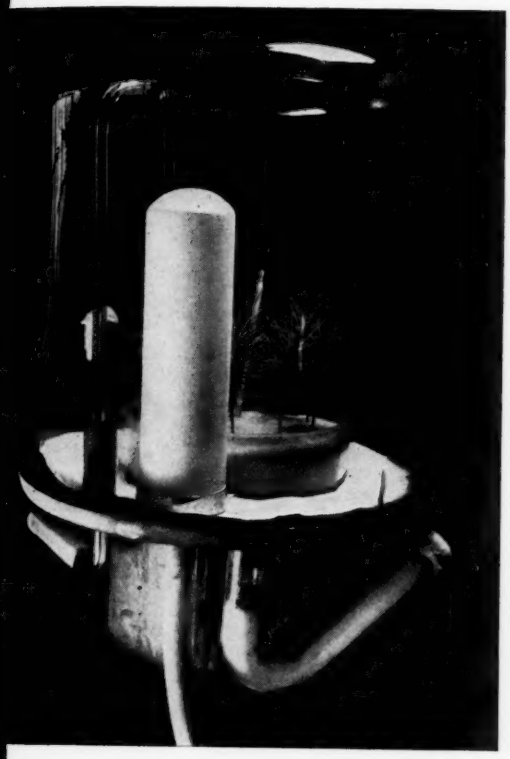

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